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Breeding and Fecundity of the Glass Shrimp, (*Pasiphaea multidentata* (Decapoda, Caridea), in the Gulf of Maine

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Apollonio, S. 1969. Breeding and fecundity of the glass shrimp, *Pasiphaea multidentata* (Decapoda, Caridea), in the Gulf of Maine. J. Fish. Res. Bd. Canada 26: 1969–1983.

About 1450 specimens of the decapod crustacean *Pasiphaea multidentata* were collected in deep waters of the Gulf of Maine in 1966–69, and were examined for size, sex, weight, breeding, fecundity, and feeding. The sizes and sex ratios of the catches vary with the egg-bearing seasons, suggesting that ovigerous females are susceptible to greater predation. The data do not permit estimates of growth rates or age of maturity. The ratios of carapace length to total length differ in males and females. Two egg clutches per female per year were produced, the second clutch being smaller in numbers, probably because of "reproductive fatigue" in the female. In each clutch the number of eggs produced is a linear function of carapace length. About 50% of the stomachs were empty. Eighteen per cent contained crustacean remains of which about half could be identified as remains of the copepods *Metridia longa*, *M. lucens*, and *Euchaeta norvegica*. Epizoic suctorians were found on a few shrimps.

Received April 15, 1969

INTRODUCTION

During a study[1] of the biology and distribution of the commercial shrimp *Pandalus borealis* Kr, about 1450 specimens of the translucent glass shrimp or ghost shrimp *Pasiphaea multidentata* Esmark were collected from some of the deeper parts of the Gulf of Maine. Although the geographical distribution of *P. multidentata* seems to be well defined, relatively little information on its biology is available.

This report presents the results of an examination into the size, sex, breeding, fecundity, and feeding of the specimens of glass shrimps that have been collected from August 1966, through January 1969, in the Gulf of Maine, and, in appropriate sections, it compares these data with previous reports on *P. multidentata*. Since the present collections were made fairly regularly throughout the year, this work adds the dimension of time to comparatively abundant material, thereby increasing our knowledge of the glass shrimp, particularly breeding and egg production and development.

METHODS

All collections were taken in 30-min tows on the bottom made with a semi-balloon otter trawl measuring 25 ft (7.6 m) on the head rope and 31 ft (9.4 m) on the foot rope. The stretch

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[1] Carried out in cooperation with the U.S. Bureau of Commercial Fisheries under the provisions of P.L. 88-309.

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mesh was 1.5 inches (38 mm) in the body, 1.25 inches (32 mm) in the codend, with a 0.5-inch (13-mm) stretch-mesh liner in the codend. The samples were preserved in about 5% formalin. Bathythermograph measurements accompanied each tow. The shrimps were measured to the nearest 0.1 mm along the mid-dorsal line of the carapace, and to the nearest 1 mm from the posterior edge of the eye socket to the end of the telson. In these specimens the latter dimension was practically equal to the total length as conventionally measured from the tip of the rostrum to the tip of the telson. The sizes referred to in this report are carapace lengths unless otherwise specified. The animals, except ovigerous females or females with clearly developing ovaries, were sexed by examining the endopods of the second pair of pleopods for the presence or absence of the appendix masculina. The weights of all shrimps in two samples were determined by allowing excess preserving solution to drain from the shrimps onto absorbent paper for several minutes and then weighing to the nearest 0.1 g. Ovigerous females were weighed with and without eggs; only the latter figure is given. The numbers of eggs on almost all ovigerous females were counted, and the stages of egg development were characterized by the system outlined by Allen (1966), as follows: stage 1, nonsegmented blastoderm; stage 2, early segmentation of the body; stage 3, eyes pigmented, abdomen little developed; stage 4, abdomen well developed and distinct, yolk reduced. The stomach contents were examined, as the condition of the animal permitted, under the binocular microscope. All shrimps were examined for symbiotes.

DISTRIBUTION

Pasiphaea multidentata is found (Sivertsen and Holthuis, 1956) in the Mediterranean Sea, the Bay of Biscay, and off southwestern Ireland, and it is common along the southern and western coasts of Norway. It occurs between Scotland, the Faroes, and Iceland, and has been taken off southeast Greenland. Squires (MS, 1965) found the glass shrimp in Cabot Strait and in Hermitage Bay, Newfoundland, and reported that it was known north of Hudson Strait, though the reference for the latter area was not given.

In the Gulf of Maine, Smith (1879), calling the glass shrimps *P. tarda* (Sivertsen and Holthius, 1956), took four specimens of *P. multidentata* in deep water east of Cape Ann, Massachusetts. Rathbun (1905) noted a few specimens off Cape Cod, and Bigelow (1926) took several glass shrimps on a number of occasions at various deepwater locations within the Gulf of Maine.

Pasiphaea multidentata has been reported from depths between 10 and 2000 m (Sivertsen and Holthuis, 1956). Elofsson (1961) took its planktonic larvae from 600 m in Korsfjord, Norway. Smith's (1879) specimens in the Gulf of Maine were taken between 203 and 311 m and Bigelow (1926) took one in a net haul from 75 m, though most came from 150 m or deeper. In each instance in Bigelow's work, the bottom depth exceeded 200 m. Squires (MS, 1965) found the species only between 200–380 m.

Figure 1 shows the locations and bottom depths of samples taken in this work. Of more than 1400 specimens taken, 13 in four samples came from bottom depths between 170 and 180 m and 52 were taken on one occasion at 194 m in Cashes Basin. All the rest were taken where the bottom was deeper than 200 m, either at about 230 m in Jordan's Basin or east of Jeffreys Ledge at about 210 m in the northern end of Wilkinson Basin. Glass shrimps were usually, but not invariably, taken in these latter two deeper basins, and were only rarely taken at other locations in depths less than 200 m.

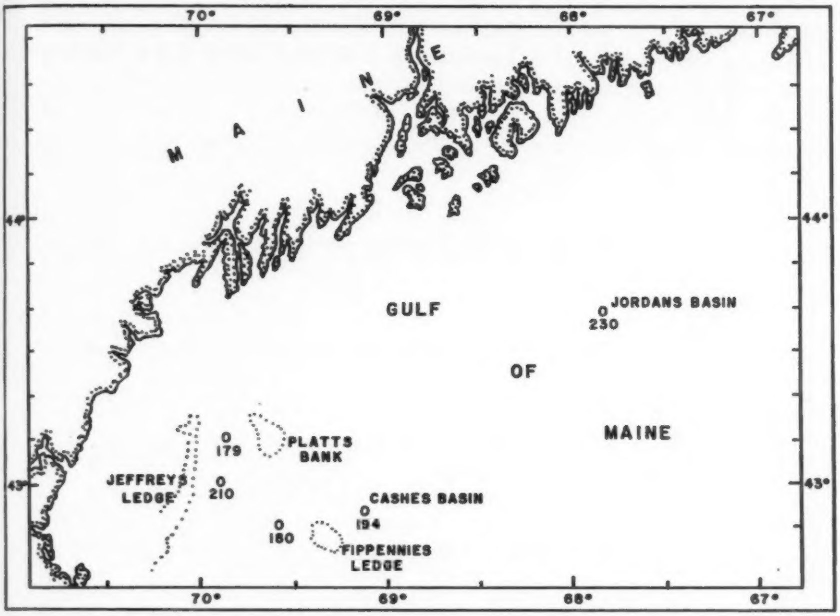

FIG. 1. Locations and bottom depths, in meters, of collections of *Pasiphaea multidentata* in the Gulf of Maine, 1966–69.

Since the net may have taken individuals on its way to and from the bottom, it is impossible to state from the available data the usual depths at which they actually live, but they probably are most abundant in depths greater than 200 m.

Grieg (1926) noted that the *Michael Sars* took glass shrimps on the Norwegian coast at temperatures between −1.07 and 8.07 C. Squires (MS, 1965) found *P. multidentata* in temperatures of 4.8–5.5 C. Assuming that all our glass shrimps were taken at 170 m or deeper (Table 1), the temperatures ranged from 4.1 to 7.3 C. About 50% of the temperatures between 180 m and the bottom were 5.0–5.5 C.

SEASONAL VARIATIONS IN SIZE AND COMPOSITION OF SAMPLES

There is considerable variation in the numbers of shrimps taken in the samples (Table 1) and the data suggest that this variation is related to the breeding season. (In Table 1 and this discussion I have omitted a total of nine shrimps contained in six samples of 1–3 nonovigerous shrimps each.) The average number of shrimps in the seven samples in which no ovigerous females were found is 13.8 (range 7–35) compared with an average of 72.5 shrimps (range 7–226) in 18 samples containing ovigerous females. The difference in means is significant at $P = 0.05$ ($t = 2.275$, 23 df).

TABLE 1. Summary of samples of *Pasiphaea multidentata* in the Gulf of Maine, 1966–69.

Date	Area	Depth (*m*)	Temp (*C*)		No. in sample	No. of males	No. of females ⩾ 18.0 mm	Nonovigerous females (⩾ 18.0 mm) w/ovarian development		Ovigerous females ⩾ 18.0 mm)	
			180 m	Bottom				No.	%	No.	%
Jan. 13, 1969	E. Jeffreys	210	–	–	35	15	18	4	22	0	0
Feb. 1, 1968	"	208	4.8	5.5	7	4	3	0	0	0	0
Mar. 6, 1968	"	208	5.1	5.2	15	9	6	3	50	0	0
Mar. 20, 1967	"	227	–	–	84	24	60	45	75	8	13
Apr. 23. 1968	"	210	5.1	5.4	13	10	1	1	100	0	0
May 15, 1968	"	201	4.8	5.5	30	10	17	9	53	7	41
Jnne 5, 1967	"	201	4.8	5.0	17	2	15	2	13	13	87
June 11, 1968	Jordans	240	5.7	5.9	174	36	102	17	17	63	62
June 12, 1968	E. Jeffreys	207	4.1	4.7	11	7	0	0	0	0	0
July 12, 1967	Cashes	194	5.0	5.0	52	26	25	1	4	16	64
July 15, 1968	Jordans	235	5.5	6.0	135	35	45	11	24	29	64
July 17, 1968	E. Jeffreys	210	4.3	4.7	29	13	10	4	40	3	30
July 27, 1967	Jordans	210	5.0	5.2	8	4	1	0	0	0	0
"	"	234	5.3	5.4	63	22	24	4	17	14	61
"	"	241	5.2	5.3	239	39	168	6	4	151	94
Aug. 16, 1966	"	230	–	–	7	2	4	0	0	4	80
Aug. 21, 1968	E. Jeffreys	207	4.4	4.8	59	19	25	0	0	24	96
Sept. 6, 1967	E. Jeffreys	223	5.3	5.3	111	38	69	2	2	63	93
Sept. 7, 1967	Cashes	207	5.9	6.0	18	7	11	2	18	7	64
Sept. 17, 1968	Jordans	238	7.2	7.3	65	6	30	0	0	16	53
Sept. 19, 1968	E. Jeffreys	201	4.8	5.0	78	40	38	5	13	25	66
Oct. 5, 1967	W. Platts	180	–	–	8	6	2	1	50	0	0
Oct. 30, 1967	E. Jeffreys	214	5.2	5.5	17	5	11	1	9	9	82
Nov. 6, 1968	"	201	4.4	4.4	28	12	6	1	17	1	17
Dec. 21, 1967	"	210	5.2	5.3	99	50	42	7	17	19	45

The sex ratio also differs with the breeding seasons. The smallest male, recognized by the presence of an appendix masculina, was 13 mm long. This is taken as the minimum size at which males and females can be distinguished and so juvenile shrimp of uncertain sex (<13.0 mm) are omitted in this discussion. Figure 2 compares the ratios of females to males in the samples with the numbers of shrimps $\geqslant 13.0$ mm taken in all the samples, and it distinguishes between samples taken with and without ovigerous females. The following points seem evident in the figure: (1) the total numbers of shrimps in the samples in the breeding season are substantially larger than in the nonbreeding season; (2) the ratio of females to males is clearly much larger in the breeding season; and (3) there seems to be a trend, in spite of the scatter, for the ratio of females to males to increase as the sample size increases.

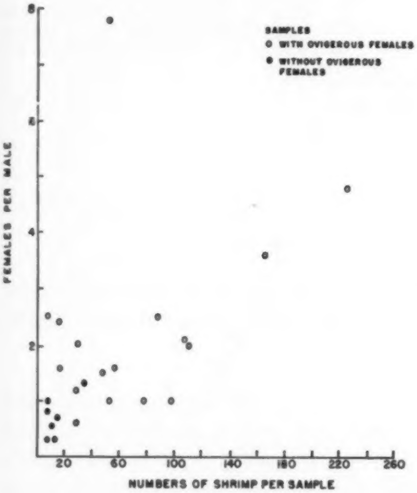

FIG. 2. Ratio of females to males plotted against numbers of *Pasiphaea multidentata* (>13.0 mm) in samples with and without egg-bearing females in the Gulf of Maine.

There is, in fact, a significantly larger per cent of females in the combined samples in the ovigerous season than in the nonovigerous season ($X^2 = 6.1$, 1 df; $0.05 > P > 0.01$).

SECONDARY SEXUAL CHARACTERISTICS

Males and nonovigerous females with undeveloped ovaries can only be readily distinguished by the presence or absence of an appendix masculina on the second pair of pleopods, but there is a measureable difference in the ratios of the carapace length to total length (i.e., eye socket to telson), as shown in Fig. 3. Normal, mature males and females with carapace lengths of 23.0 mm, for example, measure, respectively, 75.0 and 77.5 mm total length. The correlation coefficients of the regressions for males and females are 0.94 and 0.97. Allen (1962) noted such sexual dimorphism in *Spirontocaris*

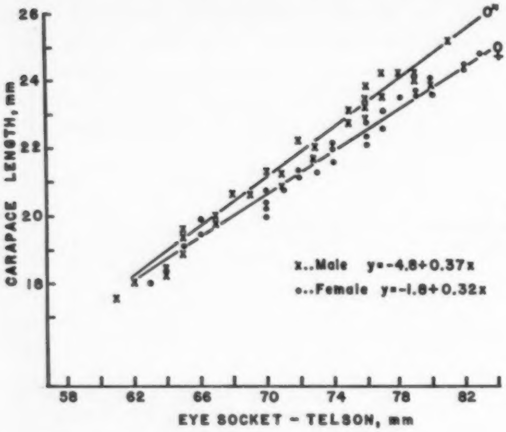

FIG. 3. Relation of total length (eye socket–telson) to carapace length of *Pasiphaea multidentata*.

lilljeborgii Danielssen in Northumberland waters, but with the total lengths of males relatively larger than those of females of the same carapace length.

SIZE AND WEIGHT

The smallest glass shrimp taken was 6.5 mm, and the largest male and female were, respectively, 30.9 mm and 29.5 mm, which are similar to those measured by Squires (MS, 1965). The smallest female ever taken with developing ovaries was 17.7 mm in July 1968, and the smallest ovigerous female was 19.1 mm taken in December 1967. The relation of size to preserved weight is shown in Fig. 4.

GROWTH AND AGE

In spite of the fact that nearly 1400 glass shrimps have been measured, and that several of the collections (Table 1) contained substantial numbers of individuals, it does not appear possible from these data to give any valid indication of their rate of growth or age of maturity. Length-frequency graphs (Fig. 5) offer little encouragement. Undoubtedly the graphs suffer from a shortage of the smaller shrimps which probably were not adequately sampled by the otter trawl. A fine-mesh midwater trawl may be the only means of making suitable collections for the clarification of the questions of growth and age.

The interpretation of length-frequency graphs may be complicated by the fact of two clutches per female in a year. A single peak might be interpreted as the combined result of all shrimps produced in both clutches within 1 year, or conversely it might represent only the results of one clutch, not both, in a year. Further, although females cannot moult and grow during either of two 5-month ovigerous periods each year, males can and probably do grow in those periods; thus there may be considerable blending and obscuring of any age-related pattern of length frequencies.

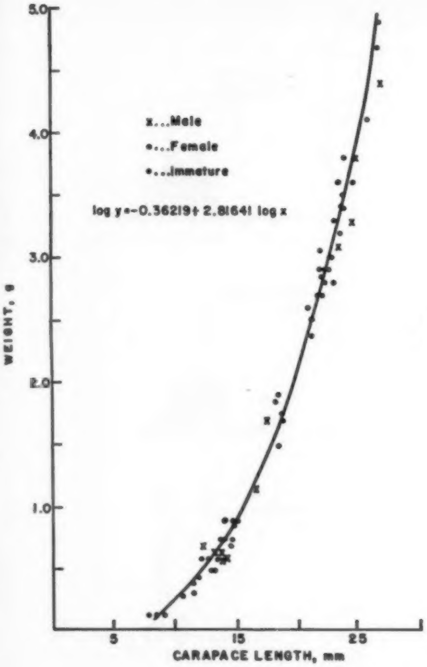

FIG. 4. Relation of carapace length to preserved weight of *Pasiphaea multidentata*.

BREEDING

Squires (MS, 1965) took a male glass shrimp with the vas deferens full of sperm in November. This study can contribute nothing of value to that limited information on sexual activity in the males. No obviously swollen semeniferous tubules were found; at best only questionably expanded tubules were noted at various seasons.

In Norway, female *P. multidentata* carrying freshly deposited eggs (Wollebaek, 1908), or eyed embryos (Grieg, 1926) have been taken in January. Both authors found highly developed embryos in March, and they observed similar early and advanced egg development in August, concluding that the glass shrimp has two breeding seasons each year.

Ovigerous females were taken in December in Hermitage Bay, Newfoundland, and Squires (MS, 1965) concluded that the glass shrimp spawned annually.

In the Gulf of Maine, Bigelow (1926) found egged females in August, but not in March or April.

Tables 1 and 2, based on the present work, summarize the breeding development of the glass shrimp in the Gulf of Maine. All the data from 1966–69 are arranged consecutively by month, without regard to the year of collection, but undoubtedly there was a difference in the time of egg production between 1967 and 1968; ovarian development and spawning apparently began later

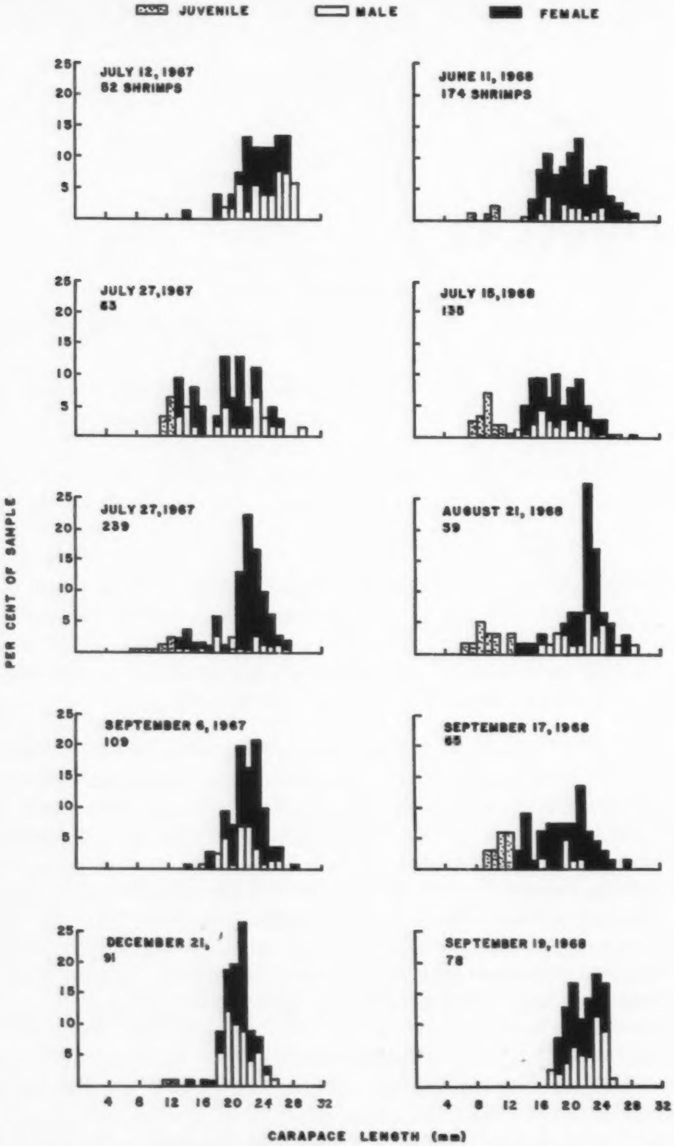

FIG. 5. Length-frequency distributions of *Pasiphaea multidentata* in the Gulf of Maine in samples of more than 50 individuals.

TABLE 2. Summary of egg development by stages in *Pasiphaea multidentata* in the Gulf of Maine by month.

Date	Ovigerous females	% of ovigerous females with eggs of			
		Stage 1	Stage 2	Stage 3	Stage 4
Jan. 13, 1969	0	0	0	0	0
Feb. 1, 1968	0	0	0	0	0
Mar. 6, 1968	0	0	0	0	0
Mar. 20, 1967	8	100	0	0	0
Apr. 23, 1968	0	0	0	0	0
May 15, 1968	7	100	0	0	0
June 5, 1967	13	93	7	0	0
June 11, 1968	63	25	67	5	3
June 12, 1968	0	0	0	0	0
July 12, 1967	16	6	75	19	0
July 15, 1968	29	52	48	0	0
July 17, 1968	3	100	0	0	0
July 26, 1967	0	0	0	0	0
July 27, 1967	14	0	65	35	0
July 27, 1967	151	8	55	34	3
Aug. 16, 1966	4	0	50	50	0
Aug. 21, 1968	24	29	71	0	0
Sept. 6, 1967	63	8	87	5	0
Sept. 7, 1967	7	0	86	14	0
Sept. 17, 1968	16	13	12	56	19
Sept. 19, 1968	25	20	72	8	0
Oct. 5, 1967	0	0	0	0	0
Oct. 30, 1967	9	0	67	33	0
Nov. 6, 1968	1	0	0	100	0
Dec. 21, 1967	19	11	53	36	0

in 1968 than in 1967. This difference may be due to cooler waters that generally prevailed in the Gulf of Maine in 1968 than in 1967. In the following discussion the breeding activity of female glass shrimps in the Gulf will be summarized by months.

We have taken only 23 females in January and February. None carried eggs at that season, but in January 1969, four had well-developed ovaries. Females in March of 1967 and 1968 showed ovarian development and in 1967 14% of the females carried eggs; all the rest showed full ovaries. No samples were taken in April and May 1967. The first egged females in 1968 were taken in May and all the other females then showed full ovaries. A few females with neither developing ovaries nor eggs were taken at various other times in the breeding season of both years; nevertheless, probably over 90% of all females over 18 mm spawn each year.

In May and June, 40–85% of females larger than 18.0 mm, apparently the minimum size of maturity, are egged. By mid-June considerable numbers of stage 1 eggs are becoming stage 2, and a few stage 3 eggs have appeared. In mid-July stage 2 eggs are found on 50–75% of all egged females, and stage

3 eggs may be on 20–35% of the females. In midsummer, June–August of 1967 and 1968, every female carrying eyed stage 3 and stage 4 eggs showed renewed ovarian development. (This ovarian development in ovigerous females is not included in Table 1.) Although females with stage 2 and stage 3 eggs predominate and are about equally common in late July and mid-August, by late August and early September stage 1 eggs again are present on 10–30% of the females, stage 2 eggs on 70–80%, and stage 3 eggs are less common than earlier in the summer. In 1967, the numbers of females with stage 3 eggs in early September were significantly less ($X^2 = 20$, 1 df; $P < 0.01$) than in late July.

All this is interpreted to mean that there are two clutches for each mature female per year. The first clutch is spawned from March through May and the larvae hatch by early or mid-August. During the later stages of egg development, the ovigerous females have renewed ovarian development. Each female then produces another clutch in late August or early September and this second clutch may hatch in December or early January. Females may thus carry the eggs of each clutch for about 5 months. Although females in July 1967 and 1968 with stage 3 eggs of the first clutch showed renewed ovarian development, none carrying the second clutch in September or later of either year did so. A few nonegged, small (18.8–21.6 mm) females in December did show ovarian development but they probably were early, first-time breeding females of the new year.

The newly deposited eggs observed by Wollebaek (1908) in January, then, were the first clutch of the year, and the eyed embryos that Grieg (1926) noted in January were perhaps the last of the second clutch of the year, as were those that Squires (MS, 1965) found in December.

The numbers of eggs were counted on nearly all the egged females collected, 297 specimens in 1967 and 158 in 1968. The eggs are rather insecurely attached to the pleopods and probably some were lost in handling, but the results are sufficiently consistent to suggest that the possible error is not serious. The results for 1967, graphed as the average number of eggs per female, for females grouped in 1-mm intervals, are shown in Fig. 6. Samples taken before and after mid-August were assigned to the first and second clutches, respectively.

Figure 6 shows that the first clutch contains more eggs than the second. This was also true in 1968, but whereas the numbers in the second clutches of 1967 and 1968 are similar, somewhat fewer eggs were produced in the first clutch in 1968 compared with 1967. I previously noted that spring spawning in 1968 was later than in 1967. Perhaps the reduced size of the first clutch in 1968 reflects that later spawning. In each instance, the numbers of eggs appear to be linear fluctuations of the carapace lengths.

Wollebaek (1908) found 50–60 eggs on glass shrimps that were 50–70 mm long. These evidently were taken from the first clutch (Fig. 6). He also mentions finding about 170 eggs on "gigantic specimens," but since these measured 140–160 mm from the telson to the tip of the rostrum, they perhaps were ***Pasiphaea tarda*** rather than ***P. multidentata***.

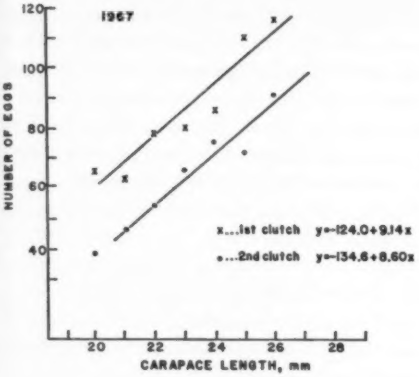

Fig. 6. Relation of carapace length to average numbers of eggs per female, for females of *Pasiphaea multidentata* grouped in 1-mm intervals.

The average size of eggs apparently does not vary between the two clutches as does the number of eggs. Stage 1 and stage 2 eggs were measured from the first clutch of May and from the second clutch of August and September 1968. The eggs were oval with dimensions of approximately 1.5–1.6 × 2.0–2.2 mm. The eggs in both clutches enlarge during development, reaching about 1.9–2.0 × 2.5–2.6 mm in stage 3 when eyes are well developed and the abdomen is apparent. These measurements agree with those given by Wollebaek (1908) and by Elofsson (1961).

Feeding

In Newfoundland, Squires (MS, 1965) found that most of the glass shrimp stomachs were empty, but remains of euphausiids, probably, were found in one stomach.

In this work 985 stomachs were examined with the results that are listed in Table 3. About 80% were empty, or contained very slight traces of unrecognizable organic material or a little mud that perhaps was picked up in the net. About 1% contained crustacean fragments, including eyes, that

Table 3. Per cent of stomach contents in 985 specimens of *Pasiphaea multidentata* in the Gulf of Maine.

Content	%
Empty	48.9
Traces of debris	18.1
Traces of mud	13.8
Crustacean debris	8.8
Copepods	7.6
Crustacean eyes (euphausiids?)	1.2
Oil droplets	0.9

could have been euphausiids. Another 9% contained crustacean fragments of unknown origin and about 8% contained clearly identifiable remains of copepods. These were identifiable fragments or nearly intact remains of *Metridia lucens*, *M. longa*, and *Euchaeta norvegica*, including ovigerous females of the latter. One stomach contained five individuals of *M. longa*. Another stomach, taken in late August 1968, was full of copepod fragments, and included five separate egg bundles, probably from *E. norvegica*. It is quite possible that the material listed as "Traces of debris" were copepod remnants, in which instance copepods are a substantial part of glass shrimps' diet.

Bigelow (1926) notes that *E. norvegica*, part of glass shrimps' diet, is found principally in the deeper waters of the Gulf of Maine, stating that 100 m appears to be its upper limit, and is most abundant in the deepest waters of the Gulf. This evidence supports the indications that the glass shrimp lives in the deepest waters of the Gulf.

SYMBIOTES

No parasites were found on our *Pasiphaea multidentata*. In June and August 1968, however, four females were found with an unidentified epizoic suctorian attached to the pleopods and a few of their eggs. One of the suctorians was producing spores.

DISCUSSION

The larger numbers of shrimps taken in the ovigerous season suggests that the shrimps are more vulnerable to capture or predation at that time. Since a relative increase in the numbers of ovigerous females accounts for much of the increase in total numbers per sample, it is probable that the egg masses impede the female swimming ability and cause the normally pelagic shrimp to sink toward the bottom or reduce their ability to avoid the bottom fishing net.

Comita and Anderson (1959) found that female calanoid copepods with larger egg numbers were taken in waters deeper than were those with fewer eggs and suggested that larger egg masses may impede swimming ability causing females to sink. Hutchinson (1967), pursuing this idea, speculated that "... excessive production of eggs might result in selection against high fecundity." Corkett and McLaren (1969) similarly suggested that "perhaps the size of the clutches is ultimately related to that which the females can carry efficiently, for example, so as to avoid predators." We can imagine that the glass shrimp, normally a pelagic shrimp, might suffer greater predation on or near the bottom, or through egg-limited mobility, and thus finds it advantageous to keep its egg numbers small and its swimming ability high. This clearly implies that in the glass shrimp, at least, selection may not favor the individuals with the largest reproductive rate, but instead that a smaller fecundity conveys survival advantage to the female and to her offspring.

Carrying eggs on the pleopods apparently subjects the females to increased risk of predation. In spite of that disadvantage, each female has two ovigerous periods per year. The survival of the species may require the total egg production contained within both clutches in spite of the risks. It is conceivable, however, that the egg mass of the first clutch could be divided into a larger number of smaller eggs, thereby attaining the total numbers required and reducing by half the period of increased predation risk. That this does not happen implies that larval success depends upon a large yolk supply for each larva. The rather large yolk reserve does result in relatively advanced larval development upon hatching; Elofsson (1961) notes that all the pereipods are biramous and the first two are chelate, and that the pleopods are biramous and functional.

Advanced development is in fact a form of parental protection and imparts to the larvae greater independence from possible food shortages, greater swimming and food-gathering abilities, and greater safety from predators.

The reproductive strategy seems to be a compromise designed to provide "parental care" to the young and to reduce predatory risks to the females. The fact of large egg sizes required for larval survival but derived from a limited egg-mass volume determined by female survival requires two ovigerous periods to produce the total numbers of offspring required to perpetuate the species.

If we assume that both clutches are necessary to produce the number of eggs required for species survival, and that the population is essentially stable over a long time period, then for a female of 22 mm, which seems to be the average size, 2/133 $\left(\frac{2}{83 + 50}\right)$ eggs (Fig. 6) will attain maturity, or the maximum survival rate is 0.15.

Thorson (1946) has reviewed several cases of decapod crustaceans, e.g. *Palaemonetes varians*, *Leander squilla*, and *Crangon crangon*, in which individual females produce more than one clutch per year. These examples are either intertidal species or are found principally inshore in rather shallow waters. I am not aware of information on the number of clutches per year in decapods from deeper waters.

A reduction in the average sizes of sequential clutches in crustaceans has been previously observed in nature, particularly in copepods (Hutchinson, 1967). That phenomenon has been attributed to two possibilities; either to an adaptation to a seasonally varying food supply for the offspring or to "reproductive fatigue" in the female.

Corkett and McLaren (1969), working in the laboratory with the copepod *Pseudocalanus minutus*, found that "the mean numbers of eggs in the sacs produced by 'healthy' females, before evidence of reproductive decline, was apparently uninfluenced by wide variations in food supply..." They also observed that "When... small sacs occur in nature they are probably indicative of a reproductive decline which is unrelated to the availability of food..."

In the case of *P. multidentata*, which appears to live as adults only in the deepest (200 m) parts of the Gulf of Maine, and whose larvae perhaps also live at relatively great depths (Elofsson, 1961) and so presumably are rather remote from the seasonal cycles of food production in surface waters, it is questionable that the clutch sizes are an adaptive response to a seasonally varying food supply for the young.

If the female reproductive capacity remained undiminished, then such a reproductive strategy, adapted to varying food supplies for the larvae, might be manifest in fewer but larger and yolkier eggs in the second clutch. In fact, the eggs of the second clutch appear to be the same size as those of the first, making that hypothesis unlikely.

More likely, the reduced second clutch may simply indicate a reduction of the female reproductive capabilities, particularly since the egg size does not vary between clutches.

A more remote possibility, related to the swimming ability and survival of the females, is that the clutch sizes are adjusted to variations in water viscosity. The larger first clutch is produced during the colder season of spring and early summer, whereas the second smaller clutch appears during the period of warming water of late summer and fall. The female, then, is relatively less encumbered in the warmer season when her swimming ability may be reduced in less viscous waters. Since the seasonal temperature variation in those deep waters is small (±1 degree C), however, the variation in viscosity may be insignificant to the female.

ACKNOWLEDGMENTS

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Demersal Fish Explorations in the Northeastern Pacific Ocean — An Evaluation of Exploratory Fishing Methods and Analytical Approaches to Stock Size and Yield Forecasts

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ALVERSON, DAYTON L., AND WALTER T. PEREYRA. 1969. Demersal fish explorations in the northeastern Pacific Ocean — an evaluation of exploratory fishing methods and analytical approaches to stock size and yield forecasts. J. Fish. Res. Bd. Canada 26: 1985–2001.

A series of demersal fish studies were conducted throughout the northeast Pacific Ocean. The aims of these exploratory fishing surveys were: (1) to describe the zoogeographic distribution patterns of the animals encountered and the relationship of such patterns to observed environmental features; (2) to determine their relative abundance in time and space and to each other and their vulnerability to sampling gears; and (3) where possible, to establish approximations of the magnitude of important or potentially important commercial species together with estimates of the physical yields that these resources might provide. A general review is given of U.S. demersal fish explorations in the northeast Pacific Ocean along with the methodology employed. Methods for estimating standing stocks based on fish densities determined from catch-per-unit-of-effort data are given. Using this procedure, estimates have been made for the more abundant demersal fish forms in the northeast Pacific. Means of determining yield possibilities are suggested, based on natural mortality rates and standing stock sizes. This method assumes that the maximum sustainable catch will range from .4 to .5 of the virgin stock size times the natural mortality. Finally the authors evaluate the reliability of exploratory fishing surveys based on subsequent development of commercial fisheries in the northeast Pacific. The data appear to have provided a good basis for describing the distribution and availability of important demersal fish elements in this area. Although estimates of yield potentials are still being studied, the history of fisheries development has, to date, followed quite well what might have been expected as a result of the preliminary yield forecasts.

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INTRODUCTION

THE TERM "exploratory fishing" has a variety of meanings. To some it is the process of scouting for commercial concentrations of fish and communicating such findings to fishermen; that is, the activity represents a search and detection arm of the fishing fleet. To others it is the process of finding suitable areas where commercial fishing can be conducted, that is, finding new banks or grounds. And to some it is the process of experimenting with the most effective means of extracting resources in a particular area.

To many scientists, however, exploratory fishing may imply a formal approach to resource assessment. In this respect it represents a study of the species complex inhabiting certain areas of the ocean, the areal and time

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distribution of the elements of the complex, their relation to environmental properties, and the relative and absolute abundance of certain segments of the fauna inhabiting a particular region. In considering exploratory fishing and its capability for providing information pertinent to industrial growth and the scientific community, this latter concept and approach is perhaps the most rewarding.

Exploratory fishing, as conducted by our Research Base, has the objective of cataloging and describing as completely as possible all the faunal elements available to certain sampling gear. The aims of the surveys are: (1) to describe the zoogeographic distribution patterns of the animals encountered and the relationship of such patterns to observed environmental features; (2) to determine their relative abundance in time and space and to each other and their vulnerability to sampling gears; and (3) where possible, to establish approximations of the magnitude of important or potentially important commercial species together with estimates of the physical yields that these resources might provide mankind.

During the past several decades rather intense demersal fish explorations with trawls, setlines, and gillnets have been made throughout much of the northeastern Pacific from the Bering Sea south to the Oregon–Washington border. In fact, when one considers the explorations in these waters by U.S., Canadian, Soviet, and Japanese scientists, one might conclude that the demersal fish fauna of this area has been more systematically and intensively surveyed than most regions of the world's oceans.

Our purpose here will be to review briefly the nature of U.S. demersal fish explorations[1] in the northeastern Pacific. Particular emphasis will be given to the survey methodology, analytical approaches to estimating standing stock sizes, and methods of making preliminary approximations of the yields per estimated standing stocks. Finally, ability of the surveys to delineate specific resources and to forecast new areas for commercial fisheries will be considered in light of those fisheries that have developed subsequent to such explorations.

BACKGROUND

Scientific studies to collect information on the marine fauna inhabiting the Continental Shelf and slope waters adjacent to the Pacific coast of the United States and Canada began in the late 1800's. The most extensive early assessment of bottom fauna in the northeastern Pacific was by the U.S. Fish Commission's steamer *Albatross* during the period 1889 through 1921. During the survey by the *Albatross*, fish and shellfish were collected with dredges, setlines, and beam trawls. These investigations greatly expanded the knowledge of species of demersal fish inhabiting these waters but yielded little information on their relative abundance or the magnitudes of populations of any species encountered. Subsequent to the studies of the *Albatross* about 20 years passed in which no major demersal fish study was made off the west coast of the United States and Canada.

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[1] In recent years the base at Seattle has carried out extensive midwater and pelagic fisheries surveys. The approaches to these surveys and data analysis differ slightly from those of demersal surveys and thus are not within the purview of this paper.

In 1940 and 1941 the U.S. Fish and Wildlife Service began a series of investigations to determine the types, distribution, and abundance of commercially important marine resources off the Pacific coast of the United States and Canada. These surveys differed from those by earlier investigators in that greater attention was directed to species that had or appeared to have potential use by man. In addition, standard commercial gear was used so that catch rates could be equated or compared with those of major world fisheries. Investigations of this nature were termed "exploratory fishing," and in the past several decades the U.S. Bureau of Commercial Fisheries and its predecessor carried out more than 40 cruises to investigate potential groundfish or shellfish resources, or both, in the waters from southern Oregon to about Cape Lisburne in the Chukchi Sea (Fig. 1). Although at times commercial

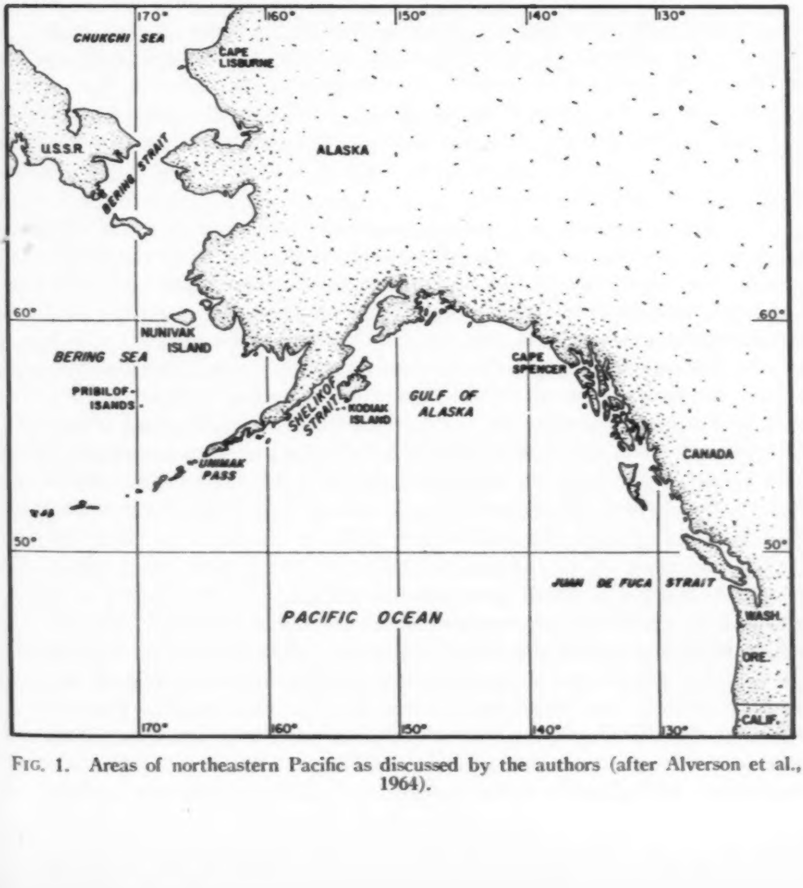
170°
160°
150°
140°
130°
CHUKCHI SEA
CAPE LISBURNE
U.S.S.R.
BERING STRAIT
ALASKA
60°
NUNIVAK ISLAND
BERING SEA
PRIBILOF ISANDS
SHELIKOF STRAIT
KODIAK ISLAND
GULF OF ALASKA
UNIMAK PASS
CAPE SPENCER
CANADA
50°
JUAN DE FUCA STRAIT
PACIFIC OCEAN
WASH.
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FIG. 1. Areas of northeastern Pacific as discussed by the authors (after Alverson et al., 1964).

boats were chartered to undertake exploratory cruises for demersal fishes and shellfishes, the exploratory fishing vessel *John N. Cobb* was used on most of these surveys. In addition the International Pacific Halibut Commission carried out investigations of groundfish available to otter trawl gear throughout much of the northeastern Pacific and Bering Sea (International Pacific Halibut Commission, 1964).

The bulk of the data obtained from exploratory surveys in the northeast Pacific has been evaluated in detail by Alverson et al. (1964) and Alverson (MS, 1967, 1968). Their analyses provide the basic documentation of the distribution, relative abundance, and magnitude of demersal fish stocks inhabiting these waters.

METHODOLOGY

Some of the difficulties in undertaking exploratory fishing activities relate to the design of a sampling program that will satisfy two primary data users — the commercial fishing industry (both the processor and producer) and the scientific community. From a scientific standpoint one wishes to approach the problem of sampling so as to most effectively determine the community or complex of animals inhabiting a particular area (i.e., identification) and at the same time to derive a fundamental understanding of their spatial distribution, how the distribution patterns change with time, and to quantify as best possible the various elements that constitute the community or animal complex.

The fishing industry, however, may have varied objectives. Although the producer, or fisherman, may desire information on long-range forecasts covering the magnitude of the resource for investment planning, he is more often interested in the distributional and extraction aspects such as where the fish aggregate, their sizes, and the availability of the resource to his fishing gear. In this regard, he wants to know how much he can catch in what period of time, where and how he should deploy his vessels to maximize the usable catch, and whether the anticipated catch is sufficient to justify his participation in the fishery. He is not as interested in total bathymetric or geographic-range of the species as he is in the bathymetric zone in which the fish tend to concentrate, the various densities of fish throughout this range, and the seasonal distribution pattern of the exploitable stock.

The processor, on the other hand, has a definite need for more general information on the seasonal geographic distribution of the stocks including areas and times of the year where maximum densities occur. He also will want to know the potential harvest and sizes of individuals from these stocks so he can plan the size of his processing operation to maximize returns. Because it is both difficult and costly to move a shore-based processing plant, information on the distribution of the resource is needed before selection of the plant site.

FACTORS GOVERNING SAMPLING ACTIVITY

Once the area of investigation has been selected, three important problems govern the actual sampling activity. They are the selection of the sampling device, the sampling pattern, and timing.

Sampling gear — The selection of the sampling device is always a difficult problem and will depend on the objectives of the investigation and the anticipated user of the information. From a scientific standpoint one might want to use the most up-to-date trawl or sampling device or a scaled-down version of a commercial trawl. On the other hand, if the equipment used is atypical to that used by the fishermen in the region, both the scientist and fisherman may have difficulty in interpreting the results because they often do not reveal the commercial potential of the stock. On the other hand there are times when largely new sampling schemes must be considered, as extant commercial systems may not reveal the resource potentials. Consequently, for almost all demersal trawl surveys in the northeastern Pacific since the 1950's, the 400-mesh Eastern otter trawl (94-ft footrope and 71-ft headrope) was the basic sampling gear (Greenwood, 1958). This trawl has been fished commercially both on the east and west coasts of the United States and, therefore, results from surveys can be more readily interpreted by the fishing industry.

Sampling pattern — The distribution of sampling for most of the demersal fish studies in the northeastern Pacific was generally governed by the character of the seabed and the known information concerning the resources being evaluated. In those regions where there were few obstructions of the seabed (i.e., sandy or mud bottom prevailed) station patterns and tracklines were established prior to particular cruises. Geographic spacing was generally fixed in a latitudinal sense (or for stations running parallel to the isobaths) but the number of stations increased where the slope gradient was steep; that is, the lines normal to the coast were mostly at fixed intervals, whereas those running parallel to the coast were largely dependent on the bathymetry of the region being investigated.

In a number of surveys in the northeastern Pacific the use of a pre-planned station pattern was not possible. This was particularly true when investigations were being made on the Outer Continental Shelf and upper continental slope where rock and coral outcroppings were common. Surveys in these areas required prior investigation of the bottom features before making decisions concerning the sampling pattern. When surveying a region having irregular bottom sediments or topography, the area was first reconnoitered with an echo sounder. Samples of the bottom were taken intermittently to determine substrate composition. The chief scientists then made detailed topographic charts of the area under study, evaluated the implied bottom characteristics, and at times made "chain drags" (i.e., attached a chain in place of the trawl between the otterboards and dragged over the bottom) to determine the suitability of the area for trawling (Hitz et al., 1961). When a number of subareas suitable for trawling had been defined in a region under investigation, subsequent sampling was planned. In many instances, however, the areas were so limited that only one or two trawl stations could be occupied. Sampling on marginal bottoms was often facilitated by placing a snag cable between the doors to prevent the nets from being damaged severely.

Timing of the sampling program — For most investigations in areas north of Juan de Fuca Strait, the station patterns were repeated in several seasons of the year. A similar approach, however, was not followed in the waters adjacent to the coast of Oregon and Washington as commercial fishing rapidly expanded into the zones under investigation. However, seasonal distribution patterns in areas where commercial fisheries operate can be evaluated by analysis of fishing catch records.

The timing of the sampling program is critical and has a pronounced influence on the results from exploratory surveys. In the northeastern Pacific, Alverson (1960) showed that most demersal species undergo extensive seasonal bathymetric movements. When surveys are restricted to one season, an incomplete understanding of the distributional patterns may result. More important, though, from the standpoint of commercial utilization are the seasonal changes in catch rates that can occur as a result of seasonal aggregations for feeding or breeding purposes. Stocks that are too dispersed during one season to be harvested economically might be profitably fished at other times of the year.

Survey Documentation

The question of what type of information should be collected during exploratory fishing cruises is one which has and will continue to plague various investigators. All too often the tendency is to program data collection far beyond the capability of satisfactory documentation by the limited staff. In the ideal situation it would be desirable to have a wide series of hydrographic observations as well as biological information on the numbers, size, weight, age, etc. of the fish being sampled. Some decision, however, based on primary work objectives must be made between what is desired and what is practical, due to staff and vessel limitations.

The fundamental data to be documented during exploratory fishing include: (1) precise identification of the area and time of sampling; (2) as complete identification of faunal elements as possible; and (3) indices of abundance. The latter will depend on accurate estimates of the weights and numbers of various animals captured.

These types of data collection are extremely important in documenting the results of exploratory surveys. Obviously, if possible, information should be collected on the age composition of the dominant species, and from such data derive preliminary estimates concerning the growth and mortality of the animals. Age data can also be a valuable clue to the probability of stability in the resource. Stomach samples are helpful in establishing feeding relationships between animals and may provide additional information on latent fish resources not sampled by the gear being used. These data may be of value in future forecasting programs or for designing further explorations. In addition, hydrographic data, particularly those phenomenon which seem to be important factors governing distribution and availability of animals, should be collected. In the surveys in the North Pacific, hydrographic data collected normally included a measure of bottom water temperature and salinity, as well as sediment characteristics of the seabed.

DATA RECORDING, STORAGE, AND RETRIEVAL

Properly conducted exploratory fishing activities, as in any scientific activity, require systematic recording of observations. Standard recording procedures are needed to ensure proper documentation of the many observations obtained at each sampling station. Hence it is desirable to develop a standard field recording form. As many observations are made during a demersal fish survey relating to the complex of species, numbers, weights, size, etc., along with hydrographic data, it is important that the records be stored in a fashion to be easily retrieved and verified.

If exploratory fishing is conducted over a number of years and throughout a large geographic area, the resource assessment activity will ultimately create a tremendous volume of data; so much that manual manipulation of the data requires a large amount of manpower and time for its effective analysis. For this reason we have developed an automatic data processing (ADP) system (Pereyra, MS, 1966) to store on ADP cards all basic information collected during demersal fish surveys (Pereyra and Barzel, 1967).

In examining the distribution and relative abundance patterns of species encountered during exploratory fishing, the data should be organized to reflect seasonal bathymetric and geographic changes (Fig. 2).[2] Usually the most striking changes in average relative abundance for a particular species will occur with changes in depth, whereas clines between adjacent areas are more difficult to define except when environmental properties or features, or both, are changing rapidly. We should emphasize that relative abundance which is most valid when intra-specific comparisons are made may be refined to examine size groups by depth or geographic clines.

STANDING STOCK ESTIMATION

The technique used to determine the standing stock and the limitations of such estimates has been discussed by Alverson (MS, 1967, 1968). The method is founded on the basic assumption that catch per unit of effort is a function of stock density in the area being surveyed and that changes in catch per unit of effort are directly proportional to changes in density (Ricker, 1940; Gulland, 1964). Knowing the speed at which a trawl moves, its sweep, etc., and making certain assumptions as to the efficiency of the gear, it is

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[2] Williams (1968) has recently analyzed a great volume of data from exploratory fishing surveys conducted off the west coast of Africa, using bathymetric and geographic zones to depict the distribution and relative abundance of animals captured.

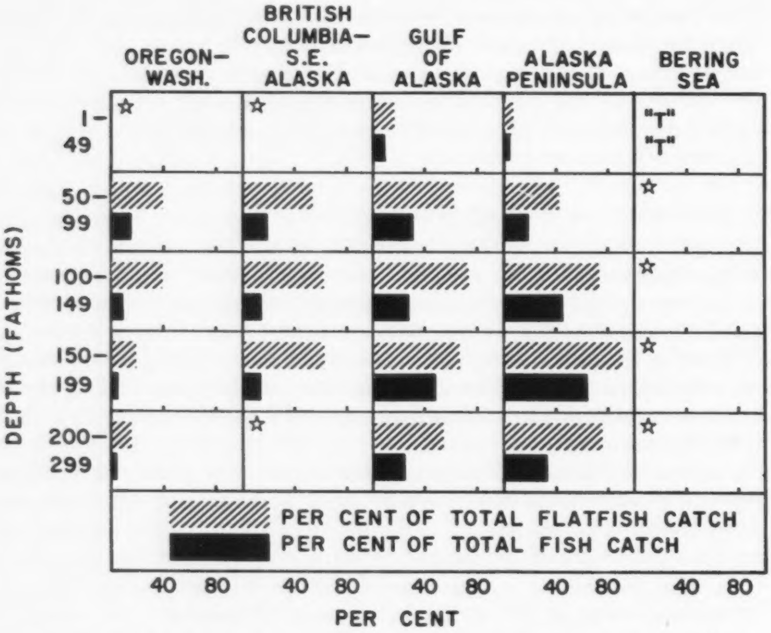

FIG. 2. Example of results from demersal exploratory survey showing the distribution and relative abundance of turbot (*Atheresthes stomias*) catches by depth and area as a per cent of total weight of flatfish and per cent of total weight of fish catches. (T = trace; * = inadequate or no sampling.) (After Alverson et al., 1964.)

possible then to calculate density and thus estimate the total population of demersal fish (of sizes susceptible to the trawl) in a defined area.

The mathematical procedures for estimating standing stock by this technique are not complicated. First, the familiar equation relating population size to catch per unit of effort is:

$$\bar{P}_{W_{ij}} = C_{W_{ij}} / qf_{ij} \qquad (1)$$

where i = ith time period; j = jth location or area; $\bar{P}_W$ = average population or standing stock in weight of the exploitable population; C_W = catch in weight; q = coefficient of catchability; f = fishing effort.

The average catch per hour for any specific depth interval or area is calculated by dividing the total catch for the interval in question by the total effort expended to make this catch. Thus

$$\bar{P}_{W_{ij}} = \sum_{k=1}^{n} C_{W_{ijk}} \Big/ q \sum_{k=1}^{n} f_{ijk} \qquad (2)$$

where the subscript k refers to the kth drag in the jth area.

With the average bottom area covered by the trawl per unit time in the *j*th area = $\bar{a}_j$ and the total bottom area of the *j*th area = A_j,

$$q = c\bar{a}_j/A_j$$

where c is a proportionality coefficient relating to the efficiency of the gear (Paloheimo and Dickie, 1964). The standing stock for the *j*th area then can be expressed as

$$\bar{P}_{W_{ij}} = \left(\overline{C_W/f}\right)_{ij} \left(A_j/c\bar{a}_j\right) \qquad (3).$$

The total standing stock for any region will be the sum of the standing stocks for the various subareas or zones forming the region.

The coefficient "*c*" is usually thought of as the coefficient of vulnerability for those fish of sufficient size to be retained by the trawl which are within the "area" swept by the trawl during a standard tow. In this definition the "area" includes the ground area covered by the trawl (distance of the tow × the spread of the net) up to the average headrope height. As such, the resulting forecast is restricted to that portion of the seabed and overlying water column that the gear can sample. For example, the standard 400 Eastern trawl used in surveys in the northeastern Pacific has an average spread during fishing of approximately 40 ft (wing tip to wing tip) and an average vertical opening of about 6 ft. The forecast must therefore be limited to the zone actually sampled by the gear and does not represent an estimate of the total population under a unit surface area of the ocean. Although it can be expected to give some preliminary minimum estimate, it is particularly subject to error in evaluating species that move well up into the water column above the seabed.

For this reason, those fish above and below the area swept by the trawl, e.g., fish buried in the seabed or those distributed farther off bottom than the headrope height of the trawl, must be considered if c is to be a true measure of vulnerability. Hence, the coefficient of vulnerability (c) as denoted in this paper will be the product of two coefficients that express (1) vulnerability of those fish that actually come within the influence of the trawl (c_h) and (2) the proportion of the total fish in the volume of water above the seabed area swept by the trawl which would come within the trawl's influence (c_v).[3] Of the two factors forming c, c_v is more difficult to estimate because it requires a fundamental understanding of the vertical density patterns of the species involved. For any of the strictly demersal species, such as flounders, which are usually close to the seabed, this value may approach 1.0. On the other hand, for those species that either burrow in the seabed and hence are below the zone of sampling or inhabit areas well above the ocean floor, c_v may have a relatively small value as a large percentage of the fish present over the area "swept" by the gear will not actually come under its influence.

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[3] c_v might be thought of as an availability term.

Under certain conditions c could be greater than 1.0, such as when demersal species are herded by the warps into the path of the trawl. Under these conditions c_h would be greater than 1.0 and c_v close to 1.0.

This may seem somewhat paradoxical and we hasten to add that it relates to one's operational definition of the coefficient of vulnerability. We have defined c_h as quantity or mass of fish caught by the trawl divided by the exploitable mass or weight of fish which was actually in the area (volume) swept by the trawl. As the area swept by the trawl is much smaller than the area under influence of the trawl doors and as the bridles from the trawl doors tend to herd certain species of fish into the mouth of the trawl, c_h will be greater than 1.0 when the herding effect results in more fish (mass) captured by the trawl than was in the area swept by the trawl prior to its passing. If c_v is near 1.0 then $c_v \cdot c_h$ (i.e., c) may exceed 1.0.

As a first approximation, one can assume that c is equal to 1.0. With increased knowledge of behavior of fish to the sampling gear and the degree to which they can escape an oncoming trawl, one should be able to forecast this coefficient more precisely. However, by assuming c to be 1.0, a preliminary forecast of the standing stock size is possible. These estimates must obviously be considered as rough approximations of the exploitable resource. They are likely to underestimate semipelagic species and could easily overestimate some flatfish populations.

Using the techniques described above and assuming c equal to 1.0, Alverson (1968) estimated the standing stocks of the 10 most abundant species in the northeastern Pacific. These estimates for various areas throughout the northeastern Pacific are given in Table 1. A similar approach has been

TABLE 1. Estimated standing stocks[a] for the 10 most abundant demersal fishes by defined areas in thousands of tons (after Alverson, 1968).

Species	Transition zone[b]	Gulf of Alaska[b]	Bering Sea[b]	Total
Yellowfin sole	–	8	2000	2008
Walleye pollack	60	130	1500	1690
Pacific ocean perch	860	370	150	1380
Rock sole	30[c]	115	1225	1360
Arrowtooth flounder	180	435	200	815
Pacific hake	750	–	–	750
Spiny dogfish	450[c]	25	–	475
Flathead sole	–	145	200	345
Pacific cod	45[c]	80	140	265
Dover sole	120	80	5	205

[a] Limited to depths less than 300 fath.

[b] Transition zone = southern Oregon to Cape Spencer; Gulf of Alaska = Cape Spencer to Unimak Pass; Bering Sea = those waters east of 175°E and south of 63°N.

[c] As sampling in the Transition zone included only a few stations at depths less than 50 fath, and as the British Columbia area was poorly sampled, the data for the Transition zone have been adjusted on the basis of estimates given by Larkin and Ricker (1964).

used by Abramson (1968) to estimate the standing stocks of pandalid shrimps off California. Edwards (1968) has also employed this method in forecasting the standing stock of demersal fishes in the northwestern Atlantic (Gulf of Maine). In his forecast he used estimates of *c* (vulnerability and availability) based on underwater television and diver observations. He also attempted to correct the estimates for the bias introduced by areal restrictions on use of the sampling gear and seasonal movements of fish in and out of the zone being surveyed.

ESTIMATING POTENTIAL PRODUCTION OF LATENT STOCKS

Several methods are now being used to estimate the potential production from biomass or standing stock figures of latent resources. The value of any such estimates, of course, relates directly to the reliability of the forecast of the biomass as well as to the assumptions that underlie the yield models employed.

A small working group established by FAO to consider world fish potentials has suggested several approaches. This group has noted that Tiurin (1962) felt that in heavily exploited fish stocks yielding catches at or near the maximum sustainable yield, F (instantaneous rate of fishing mortality) generally is equal to or exceeds M (instantaneous rate of natural mortality). If this relationship generally holds, then one can make first approximations of yield by either the Schaefer or Beverton and Holt models. We have chosen to refer to these yields as yield per exploitable biomass (YEB) rather than maximum sustainable yield. Although YEB may closely approximate the theoretical maximum sustainable yield (Ricker, 1958), these two yield estimates are not necessarily similar as the calculation of YEB involves standing stocks which are based on the catch rate of the size groups selected by the sampling gear. The term YEB, however, may be more functional in that it implies variable yield possibilities with time and can be easily related to the economic usable portion of the surveyed populations.

By the simpler Schaefer model, the maximum yield C_{max} is achieved when the exploitable population reaches approximately one-half its original or virgin biomass B_o. Assuming $F \approx M$, first approximation of the maximum sustainable catch would be: $C_{max} = 0.5\ M\ B_o$. If the yield tables from Beverton and Holt are used and if we assume that we again can extrapolate from the virgin state to heavy fishing, yield can be expressed as a proportion of the virgin population. For the Beverton and Holt constant parameter model the relationship of C_{max}/B_o can be calculated provided age at first capture is specified. The value differs slightly depending on age of first capture and also the values of (M and K) but over the most likely range of these parameters C_{max}/B_o does not differ much from 0.4 M. Thus we may use as a rough approximation the relationship $C_{max} = 0.4\ M\ B_o$ (J. A. Gulland, personal communication). Hence estimates based on either model will be similar.

If the above relationship between natural mortality and expected yields from known population sizes holds, we can expect most of the demersal fishes (which have relatively low natural mortality rates) sought by the major trawl fisheries of the North Atlantic and Pacific to provide yields which are a small fraction of the virgin biomass (e.g., $\mathrm{YEB} < .20\ \mathrm{B}_o$). For species having high natural mortality and a rapid flux of material as in some tropical forms, relationships between YEB and B_o may be much closer, and in extreme instances it is possible for the yields to exceed measured standing stocks, i.e., when $\mathrm{M} > 2.0$. The latter is common for many plankton forms but not for commercially exploited fishes.

Table 2 provides estimates of the expected range of YEB for the 10 most abundant demersal fishes in the northeast Pacific along with maximum and average catch for the highest 5 consecutive years. In calculating YEB the natural mortality M for most of the species was considered to be somewhat greater than 0.2 but less than 0.4. Hence the maximum yield ranges are between 0.2 and 0.4 times one-half the virgin stock size. Those fisheries which have "peaked" and catches subsequently declined are indicated by an asterisk (*).

TABLE 2. Estimated yields per exploitable biomass from the 10 most abundant commercial usable demersal fish and harvest level for specified years in thousands of tons (estimates limited to depth of 300 fath).

Species	Estimated YEB	Catch (highest)	Year	Catch high 5 consecutive years (avg)	Years	Catch 1966
Yellowfin sole	201–402	550	1961	340	1959–63	140[a]
Walleye pollack	169–338	260	1966	130	1962–66	260
Pacific ocean perch	138–276	470	1965	271	1962–66	315[a]
Rock sole	136–272	8	1964	4	1962–65	8
Pacific hake	150–270	175	1967	150	1966–67 only	130
Arrowtooth flounder	82–164	39	1964	22	1962–66	18
Flathead sole	35–70	11	1964	9	1962–66	5
Dover sole	21–42	9	1963	7	1960–64	4
Pacific cod	27–54	42	1966	27	1962–66	42
Spiny dogfish	20–30	67	1944	37	1941–45	4[a]

[a] Fisheries which have peaked and subsequently sharply declined.

These forecasted yield potentials based on standing stock size of any particular species represent biological potentials and not necessarily the realizable yields. They are the maximum expected yields if the population stocks could be properly and effectively used. This is seldom the case. Fisheries are not based on average geographic catch rates but operate only in those regions where catch rates are sufficiently high to allow the producer to make a profit from his operation. In many areas and depths the density of a species may never reach high enough levels to allow economic extraction. Hence the economic potential represents an extractive capability within or equal to the biological potential.

If estimates of the rate of natural mortality for the species under investigation are not available, one might substitute the rate of natural mortality of a closely allied species. If the life histories of the two species are similar, this approach may represent a suitable first approach for estimating yields. As information begins to accumulate on the various species being surveyed, attempts should be made to establish specific rates of growth and natural mortality for more abundant forms. If these two biological parameters can be established, yield per recruit curves can be derived along with more sophisticated forecasts of the maximum sustainable yield.

One such simulation approach for estimating the equilibrium yields to be expected from virgin populations off Oregon and Washington was employed by Pereyra and Tillman (MS, 1966) for Pacific hake and Pacific ocean perch. In their study, calculations were carried out using the "Piece-wise Integration of Yield Curves Program — FRG 708" written by the Fisheries Research Institute of the University of Washington and described in detail by Paulik and Bayliff (1967). In this program approximate yield isopleths are calculated after the exponential equilibrium yield model of Ricker (1958). All instantaneous rates (natural mortality, fishing mortality, and growth) for Pacific hake were age specific, being derived from exploratory fishing data. Similar data for the Pacific ocean perch were computed using vital statistic data given by Westrheim (MS, 1950). Yield was varied by changing the age at first capture in the fishery and applying multipliers to all the individual age specific rates of fishing mortality to vary the total fishing mortality. A biomass vector was obtained giving the relative weight of the stock at each age in the absence of fishing. By combining this relative biomass vector with the estimate of standing stock of the virgin population obtained from analysis of exploratory survey data, the total weight of each year-class composing the standing stock was calculated. Back-calculating from knowledge of the selectivity of the sampling gear provided an estimate of weight at recruitment which could then be used to calculate the equilibrium yields in the usual manner.

ABILITY OF SURVEY RESULTS TO PREDICT THE DEVELOPMENT OF MAJOR FISHERIES

Reliability of data collected during exploratory surveys may be evaluated against their overall objectives; that is, how well do such surveys (1) identify the faunal components and properly identify the dominant species or important fish or shellfish constituents in the area being surveyed; (2) indicate the annual and seasonal distribution patterns of the animals within particular geographic regions; (3) portray the distribution of mass of various species identified; and (4) provide data useful for judgments in the development and management of fisheries.

From a study of several thousand exploratory trawl stations occupied in the northeast Pacific, Alverson et al. (1964) identified 55 species of demersal fishes and several genera as being common components in the trawl stations occupied throughout the northeast Pacific. When these data are compared with the zoogeographic literature in existence prior to the surveys, one finds that practically all species (except for some rare specimens), which had been previously reported by areas and depth zones, were encountered. In fact many new zoogeographic and bathymetric distribution records were established as a result of the investigations.

Exploratory surveys in the northeast Pacific were almost all completed before the development of major commercial fisheries; hence we may examine these data in relation to catches from commercial fishing that developed subsequent to the surveys. The first large-scale trawl fishery to develop in the northeast Pacific occurred in the Bering Sea. This fishery began in the late 1950's, and now the eastern Bering Sea is the major trawl fish zone of the northeast Pacific. The 10 species listed by Alverson (1968) as being dominant animals in the region's surveys (Table 3) now represent the dominant forms harvested by commercial trawl fisheries in the regions. In order of importance the fisheries have varied from year to year, but in general, yellowfin sole (*Limanda aspera*) and walleye pollock (*Theragra chalcogrammus*) have dominated the Bering Sea catches, with Pacific ocean perch (*Sebastodes alutus*), Pacific cod (*Gadus macrocephalus*), arrowtooth flounder (*Atheresthes stomias*), flathead sole (*Hippoglossoides elassodon*), and rock sole (*Lepidopsetta bilineata*) being taken in lesser quantities. There are no major catches of demersal species that were not portrayed as dominant elements in the fauna by exploratory survey. In fact, the two dominant species being exploited were ranked first and second in the Bering Sea resource surveys.

TABLE 3. Ten most abundant species[a] by defined areas (after Alverson, 1968).

Transition zone[b]	Gulf of Alaska	Bering Sea
Pacific hake	Arrowtooth flounder	Yellowfin sole
Pacific ocean perch	Pacific ocean perch	Walleye pollack
Spiny dogfish	Flathead sole	Rock sole
Arrowtooth flounder	Walleye pollack	Flathead sole
Dover sole	Rock sole	Pacific cod
Lingcod	Pacific cod	Pacific ocean perch
Sablefish	Dover sole	Arrowtooth flounder
Walleye pollack	Sablefish	Starry flounder
Pacific cod	Pacific halibut	Pacific halibut
Ratfish	Starry flounder	Sablefish

[a] Limited to depths less than 300 fath.

[b] As sampling in the Transition zone did not include stations at depths less than 50 fath, the data have been adjusted on basis of the local fisheries in this area and on the stock estimates given by Larkin and Ricker (1964) for the British Columbia area.

In the waters south of the Aleutians and Alaskan Peninsula through the arc of the northeast Pacific, exploratory surveys indicated that Pacific ocean perch was the dominant species inhabiting the Outer Continental Shelf and upper continental slope (100–200 fath). Subsequent fisheries by the Soviets and Japanese in these waters has substantiated these observations, not only as to the relative abundance of species along the Continental Shelf but also to the general bathymetric region in which the species would be exploited. The results of all commercial fishing tend to reflect close correspondence with the forecasted distribution and abundance by general areas and depth zones as given by Alverson (1968). In the waters off Oregon and Washington, exploratory data suggested that the Pacific ocean perch was the dominant element on the upper continental slope, whereas Pacific hake was the most important demersal-pelagic species inhabiting the shelf waters. These data have also been subsequently confirmed by Soviet investigations and fisheries development.

Ronholt's (1963) analysis of exploratory surveys for pandalid shrimp illustrates how well such data forecast distribution of mass, dominant species, and the probable regional development of commercial fisheries. His forecast has since been confirmed by Soviet studies (Ivanov, 1963), but perhaps of more importance, closely forecasted areal and bathymetric zones from which important commercial yields have been taken in the Kodiak and Shumagin Island areas.

In each region where commercial fisheries have developed subsequently and where a broad range of the species is exploited, the distribution of fishing and catches has been consistent with projections of exploratory fishing studies. The characteristics of these fisheries do not necessarily verify the correctness or precision of estimated standing stocks or YEB's, but trends in catches to date in no way invalidate the forecasts; to the contrary, they suggest these estimates are in the proper "ball park" (Table 2). Hence activities of this type, if properly conducted and documented, can provide an index of the distribution patterns and abundance of species likely to be encountered.

The development of commercial trawl fisheries in the northeast Pacific subsequent to exploratory trawl surveys supports our opinion that assessment of latent resources represent a good basis for judgment concerning development of fisheries, including the deployment of effort, strategy of fishing, and perhaps even the proper level for capitalization. We are convinced that the preliminary resource assessment data can also be used as a guide to management needs. These surveys can document the character of resources prior to man's intervention including reliable information on natural mortality and growth of various species. First approximations can also be generated concerning the size of the stock and the yields that might be anticipated. Of course, the yield estimates should be employed only with a full understanding of their susceptibility to normal sampling error and the assumptions which underlie the estimates.

Once extensive fisheries have developed in an area, properly conducted exploratory re-surveys can be a valuable addition to data being received from the fishery to aid in management of the resources. This can be particularly valuable in making pre-season forecasts (Abramson, 1968), year-class strength estimates, or in examining stocks or those parts of the stocks outside the area being commercially fished. Also, with thepresent high mobility of to day's high seas fishing fleets, quite often classical management models are not feasible due to the unsteady state of the fisheries. Under these conditions a continuing survey program is an alternate method for obtaining current information on the stocks to permit meaningful management decisions to be made by regulatory bodies.

More sophisticated scientific investigation obviously will permit refinement of these preliminary forecasts and estimates. Nevertheless the estimates can serve as a basis for a rational approach to management problems. In fact, at times one wonders whether management decisions at an early stage of fisheries development based on the analysis of exploratory survey data might not lead to a more stable and economically viable fishery than a fishery that follows the normal course of boom and bust, followed by a parade of scientific post mortems.

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Epibenthic Algal Production and Community Respiration in the Sediments of Marion Lake[1]

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Gross epibenthic algal production and benthic community respiration in Marion Lake, British Columbia, were measured during 1968 by following changes in dissolved oxygen over undisturbed sediment cores. No measurable chemical uptake of oxygen occurred during short-term experiments. Multiple regression analyses showed that in situ oxygen production by epibenthic algae was directly related to temperature, light, and community respiration and inversely related to day length. Mean weekly values of these variables were substituted into the regression to estimate annual gross algal production on sediment at various depths in Marion Lake. Photosynthetic efficiency ranged from 0.4 to 3.1% and increased with depth of water over the sediment. Sedimentary chlorophyll was stratified with highest concentrations in the upper few centimeters of sediment which corresponded to the depth of oxygen penetration.

Measurements of in situ oxygen consumption showed that community respiration was related to temperature, oxygen concentration, and day length in a curvilinear manner. Oxygen uptake was minimal at midday and increased during the night. A multiple linear regression was derived, after suitable transformations, and mean weekly values of variables substituted to estimate annual community respiration.

Bacterial respiration was measured as the difference in total community respiration when antibiotics were added to water over undisturbed sediment cores. Less than 30% of community oxygen consumption was inhibited by antibiotic treatment during the summer, whereas over 45% reduction occurred during the winter. Bacterial respiration was directly related to temperature and showed no significant correlation with oxygen concentration or other variables shown to affect community respiration. Macrofauna respired 33% of the total oxygen consumed by sediment cores during June, and epibenthic algae were estimated to account for 23% of community respiration. Net epibenthic algal production, calculated by correcting gross oxygen production for estimated algal respiration, was 85% of gross production during the summer.

Annual carbon flux across the sediment in Marion Lake was estimated by comparing processes of carbon addition and removal. From previous studies, phytoplankton and macrophytes added 0.8 and 18 g C m^{-2} $year^{-1}$. Organic particulate matter from the inlet stream, other than macroscopic debris, contributed 143 g C m^{-2} $year^{-1}$ and average gross epibenthic algal production was 40 g C m^{-2} $year^{-1}$. Community respiration consumed 57 g C m^{-2} $year^{-1}$. An additional 143 g C m^{-2} $year^{-1}$ was lost as particulate matter in the outlet stream and emerging insects could remove 1.3 g C m^{-2} $year^{-1}$. Only 8 g C m^{-2} was estimated to accumulate below the aerobic surface–sediment layer annually.

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INTRODUCTION

PREVIOUS STUDIES in Marion Lake, British Columbia, have shown that both the standing crop and production of phytoplankton may be lowered by flushing (Dickman, 1969). This appears to be at least a partial explanation for the low rates of phytoplankton primary production observed in Marion Lake by both Efford (1967) and Dickman (MS, 1968). Benthic invertebrates, however, are extremely abundant (Hamilton, MS, 1965) and production rates of the two amphipod species in the benthic community are very high (Mathias, MS, 1967) relative to primary and secondary production in the water column. Resident fish and salamander populations in Marion Lake rely almost exclusively on bottom fauna, although organisms are occasionally taken from the water column (Hamilton, MS, 1965). These observations illustrate the importance of the benthic community in the lake ecosystem and indicate the necessity for detailed studies of energy transfer within the sediments if a complete description of energy flow through Marion Lake is to be realized. This paper describes initial attempts to quantify some processes of carbon addition and removal in the sediments of Marion Lake.

Carbon flux across the mud–water interface was measured by changes in oxygen concentration over sediment cores and by converting these measures to carbon by appropriate photosynthetic and respiratory quotients. Oxygen consumed by sediment cores placed in the dark provided a measure of total community respiration, whereas oxygen production by cores exposed to light was used as an index of epibenthic primary production. Similar methods have been used by other workers to measure oxygen production and consumption by benthic communities (Pamatmat, 1968). The approach has the advantage that autotrophic and heterotrophic processes are measured and compared simultaneously.

Total oxygen production by epibenthic algae was measured to quantify this source of primary production in the benthic community. Oxygen uptake was partitioned among bacteria, algae, micro-, and macrofauna to evaluate the relative importance of each group in total community respiration. These results were converted to carbon and combined with measures of macrophyte and phytoplankton production and estimates of other processes which add and remove carbon in the benthic community. These observations provide a framework for further detailed studies of energy transfer within the sediments of Marion Lake.

MATERIALS AND METHODS

Intact sediment cores were taken from Marion Lake, a small (13.3 ha), shallow (mean depth 2.2 m) lake located in the Coastal Mountains in southwestern British Columbia. Maximum depth of the lake is 6 m but this may reach 7 m during spring and fall peak run-off. Water temperature ranged from 3 C in January to 25 C in July 1968. The water column was generally well mixed, although an unstable thermocline formed at 1.5 m during the summer. Deep silt-ooze sediments (gyttja), covered with an algal mat and stabilized by chironomid tubes, cover the entire lake bottom except near the inlet and outlet streams where gravel is exposed. Sediment and water *p*H ranged from 6.4 to 6.9 during 1968. Further details are reported by Efford (1967).

In preliminary experiments with sections of sediment cores, 75–85% of the macrofauna was found in the upper 2 cm of sediment. In similar sections prepared for direct cell counts by methods used for soil samples (Jones et al., 1948) algal and bacterial cells were also most abundant in the upper 0.5 cm. A sediment sampler was designed to sample such a stratified community which ensured an undisturbed mud–water interface (Fig. 1). The soft texture of the sediment and the shallowness of the lake allowed the sampler to be pressed a constant distance into the mud without disturbing the interface. The hinged lid closed as the sampler was pulled up, thus preventing stirring of the sediment by turbulence. This also provided sediment with overlying water from which oxygen samples could be taken.

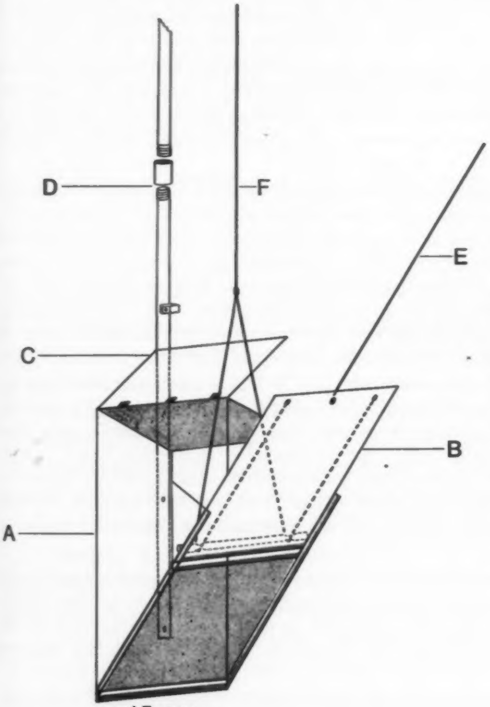

FIG. 1. Sediment sampler, constructed from ¼″ plexiglass, designed to take undisturbed samples of soft sediment and overlying water: (A) open ended box (*shaded*) with lower end cut diagonally and edges bevelled to facilitate penetration into sediment; (B) sampler bottom; (C) top, hinged along one side, open when sampler lowered into sediment, closed when sampler pulled upwards; (D) sections of aluminum pipe, coupled at 2-m intervals. Ropes (E) and (F) used to hold open and close the bottom of the sampler.

The sampler was modified to take specific layers of sediment for chlorophyll analyses and cell counts. Thin trays, slid through gasket-sealed slots in the side of the sampler after it was filled with sediment, divided the sample into 1-cm layers. Smearing was minimized by bevelling the cutting edge of the trays. By opening the diagonally closing sampler bottom, sediment could be removed below the bottom tray and successive layers collected.

Pyrex glass cylinders (12.5 cm long, 5.0 cm diam) were used to take three cores (each 20 cm^2) from the undisturbed mud brought to the surface in the sampler. The cylinders, bevelled at the bottom, were rotated as they were pushed 5–6 cm into the sediment to prevent distortion of surface sediment. The overlying water was undisturbed and completely filled the upper half of the cylinder. By placing a large rubber stopper flat over the top, thus avoiding air spaces, it was possible to partially withdraw the core and place a rubber stopper in the lower end. The upper stopper was then pushed aside and the core treated as described below.

Oxygen production by epibenthic algae was measured in clear glass cylinders. A watch glass held in place by an elastic band covered the top. Oxygen uptake by the total benthic community was measured in cylinders which had been painted and taped to ensure darkness. A rubber stopper, similar to that in the bottom of the cylinder, sealed the upper end. Oxygen uptake by bacteria was calculated by comparing respiration in antibiotic-treated and untreated sediment cores. Antibiotic solutions of streptomycin (SO_4) and neomycin were prepared immediately before use at concentrations equivalent to 50 mg liter^{-1}. These concentrations were sufficient to prevent oxygen consumption by suspensions of bacteria cultured from the sediment and bacterial growth, on a caseinate medium (Hayes and Anthony, 1959), was halted for up to 4 days. Neither amphipod respiration nor C^{14} uptake by an axenic culture of *Navicula* were affected by these antibiotic concentrations.

During production and respiration experiments, two clear cylinders and one dark cylinder which contained sediment cores were placed back in the lake at the location of sampling. Duration of incubation varied from 1 to 3 hr depending upon light and temperature conditions. Changes in oxygen concentration of 10–20% of the total oxygen present were sufficient for accurate measurement.

Incident solar radiation (cal cm^{-2} min^{-1}) was continuously recorded on a Belfort Recording Pyrheliograph. Daily photosynthetic energy at the lake surface was calculated by determining the area under the inked record, converting this to cal cm^{-2} and assuming that 50% of the total radiation was available for photosynthesis (Ryther, 1956). An underwater photometer (F. Schueler, Waltham, Massachusetts) with an opal filter (maximum absorption 5600 mμ) was calibrated against the pyrheliograph throughout the day on several occasions during 1968. This relationship changed significantly during the year and the per cent total radiation reaching the sediment at various depths was calculated at various depths during the summer and winter.

Changes in amounts of dissolved oxygen in water over sediment cores were measured by a semimicro-Winkler technique (Fox and Wingfield, 1938) with phosphoric acid used to acidify. The oxygen concentration of two 5-ml aliquots taken from over the sediment before cores were taken was compared with similar samples from the cylinders after incubation. Since oxygen concentration gradients can develop in water enclosed over sediment cores (Carey, 1967), water in the cylinders was slowly mixed during filling of the syringe. A thiosulphate solution of .0025 N and a 5-ml microburette allowed titration volumes (2–3 ml) to be accurately estimated (standard error ±2% on replicate samples).

The water remaining over sediment cores, usually about 100 ml, was measured in a graduated cylinder and changes in oxygen concentration were expressed as milliliters O_2 m^{-2} hr^{-1} at in situ temperatures. Total oxygen production was calculated by adding oxygen consumed in the dark cylinder (total community respiration) to net changes in oxygen in cylinders exposed to the light.

To determine oxygen consumption by macro-invertebrates separated from sediment, animals were placed in 5-ml syringes filled with membrane-filtered (Millipore, HA, 0.45 μ pore diam) lake water. Syringes without animals served as controls. After 6–10 hr at 15 C, depending upon the size and number of animals present, dissolved oxygen was measured as described above, with corrections for animal volume. Changes in absolute amounts of oxygen in experimental and control syringes were expressed as μliters O_2 animal^{-1} hr^{-1}.

Oxygen uptake by rotifers and ciliates was calculated from an extrapolated regression curve relating body size to respiration rate and an estimate of the number of individuals present. Respiration by epibenthic algae was calculated by difference when oxygen uptake by all other groups in the community was totalled and compared with total community respiration.

No significant changes in dissolved oxygen occurred in water-filled cylinders without sediment or over sediment cores treated with 10% neutralized formalin. All changes in dissolved oxygen are thus assumed to be due to biological activity within the sediment. Cylinders were frequently washed in hot sulphuric acid–dichromate oxidant to remove accumulated organic surface film.

Sedimentary chlorophyll was measured as an index of epibenthic algal standing crop by a modification of the technique described by Vallentyne (1955). Wet sediment, approximately 2 g, was weighed into glass-stoppered centrifuge tubes and extracted with 15 ml 90% acetone which contained 0.5% redistilled dimethylaniline and 1 drop of 1 N HCl. Undegraded chlorophylls are converted to their corresponding phaeophytins by the addition of this acid. A similar sample was weighed, dried (50 C) for 24 hr, and reweighed. This drying temperature was chosen on recommendations given by Lovegrove (1966) to avoid loss of volatile organic substances. Although some water may remain at these temperatures all samples were treated similarly and are thus comparable. Water content could then be calculated and sedimentary chlorophyll expressed on a dry weight basis without drying the sample used for analysis.

After 12 hr extraction in the dark at 5 C, the sample was centrifuged and the supernatant collected in a graduated cylinder. The remaining sediment was repeatedly extracted with clear 90% acetone until no colour was visible in the filtrate. The volume of the filtrate was recorded and the optical density of an aliquot measured with a Perkin-Elmer spectrophotometer at 667 mμ in a 1-cm quartz cell. Acetone and dimethylaniline served as a blank. Since one sedimentary chlorophyll unit, dissolved in 10 ml of solvent and read in 1-cm cells, gives a density reading of 0.10 (Vallentyne, 1955):

$$\text{Sedimentary chlorophyll} = \left(\frac{\text{density reading} \times \text{filtrate volume } (ml)}{\text{dry weight extracted sediment } (g)}\right) = \text{units g}^{-1}$$

RESULTS

GROSS EPIBENTHIC PRIMARY PRODUCTION

To estimate annual primary production of epibenthic algae on the sediment, the effect of several factors which could affect measured rates of oxygen production were examined. Eight stations, arranged along two transects, gave a range of depths of water (0.5–6.0 m) over the sediment representative of the total depth profile of the lake. Samples were taken at least once per month at all stations for 1 year. Total oxygen production for all measurements from each station were compared with incident light and temperature at the sediment surface during incubation, total community respiration (discussed below), day length, and oxygen concentration, by using a step-wise multiple linear regression. Various transformations of variables did not improve the regression and thus all relationships are assumed to be linear (Table 1).

This regression analysis allowed comparison of oxygen production on a standard basis and allowed the evaluation of the relative importance of various factors which might affect benthic primary production. This is indicated in Table 1 by the accumulated MR^2 values. Temperature was the most important variable affecting oxygen production, whereas light was secondary. It must be noted, however, that temperature and light are themselves correlated since data included in the regression were collected during an entire year. Pomeroy (1959), in a similar study, also found temperature-dependent photosynthesis in algae on a Georgia salt marsh. He attributed this to an optimal light intensity for photosynthesis (1.2–13.6 cal cm^{-2} hr^{-1}), as calculated from Ryther (1956), since photosynthesis is temperature-dependent within these light intensities (Rabinowitch, 1945–56). Incident light reaching sediments in Marion Lake was within this range, except in shallow water during the summer and in deeper water during the winter. There was no

TABLE 1. Multiple regression analyses of various dependent and independent variables considered to affect oxygen exchange across undisturbed sediment cores taken from Marion Lake during 1968. *b* is the slope constant and SE the standard error of the slope coefficients. MR^2 indicates the accumulated variation (as per cent) of the dependent variable accounted for by the step-wise entry of independent variables. All coefficients significant at $P < 0.05$.

Dependent variable:	Gross oxygen production ($ml\ O_2\ m^{-2}\ hr^{-1}$)			$\log_e$ Community respiration ($ml\ O_2\ m^{-2}\ hr^{-1}$)		
Independent variable:	*b*	SE	MR^2	*b*	SE	MR^2
Temp (*C*)	1.49	0.229	67.8	($\log_e$) 0.112	0.008	71.0
Light ($cal\ cm^{-2}\ hr^{-1}$)	0.55	0.062	76.2	–	–	–
Oxygen concentration ($ml\ O_2\ liter^{-1}$)	Not significant			($\log_e$) 1.46	0.204	73.0
Community respiration ($ml\ O_2\ m^{-2}\ hr^{-1}$)	0.83	0.134	81.5	–	–	–
Time of day^2 $(hr/100)^2$	Comparison not made			0.0034	0.0010	74.8
Time of day (*hr/100*)	Comparison not made			–0.076	0.0290	76.4
Day length (*hr of light*)	–1.66	0.567	83.0	0.023	0.0226	76.6
Y intercept	9.25			–2.39		
No. of observations	129			222		

evidence of light inhibition even at the highest light intensities (0.75 cal cm^{-2} min^{-1}).

There was a significant relation between community respiration and gross oxygen production. Kajak and Rybak (1966) also reported a positive correlation between the abundance of phytomicrobenthos and macrobenthos in several Masurian lakes. These data suggest a close temporal and spatial relationship between algal production and utilization by the remainder of the benthic community. Work currently in progress has shown that the deposit-feeding amphipod *Hyalella azteca*, by digesting algae and bacteria from ingested sediment, may stimulate microflora production. Additionally, spatial differences in epibenthic primary production are significantly correlated with amphipod abundance.

Day length, a function of season, was inversely related to epibenthic algal production, once variation due to temperature, light, and community respiration was removed (Table 1). Thus, although gross oxygen production was greater during the summer, owing to increased light and temperature, it was lower than would have been expected on the basis of these variables at other times of the year. This may reflect the grazing pressure of benthic invertebrates which have maximum growth during the summer (B. T. Hargrave, unpublished data).

Recent work (Dr G. Greundling, personal communication) has shown that algal standing crop and species composition in the sediment changes seasonally and with depth in Marion Lake. Thus, some of the effects of the variables considered to affect oxygen production in the present study may be related to algal standing crop. No continuous measure of algal biomass was attempted, except for cell counts at different depths in sediment cores. Chlorophyll analyses and visual observations of surface sediment, however, showed increased algal standing crop during the summer. Thus, some of the significant effect of day length, and other variables, on oxygen production could be due to changes in algal biomass.

Spatial differences in sediment oxygen production were very small (±10%) as estimated by incubating 12 replicate sediment cores simultaneously. Oxygen production decreased with increasing depth of water over sediment, as would be expected on the basis of reduced light and temperature with greater depth. This occurred over the entire lake except near springs where production was 40% below that observed 8 m away at the same depth. Production of oxygen by epibenthic algae within patches of macrophytes (*Nuphar* sp. and *Potamogeton* sp.) was 30–50% below similar measurements on nearby unshaded sediment. All such variation could be related to changes in light and temperature. Thick *Mougeotia* sp. mats which occurred in shallow water around the periphery of the lake were not considered in these production measurements. Undisturbed cores could not be taken because of the filamentous nature of the mats.

Vertical stratification of the algal community within the sediment was apparent from preliminary cell counts. Cells with intact chloroplasts were found only in the upper 3 cm with most cells in the 0.5-cm layer. Light penetration into sediment was estimated by covering the photocell of the underwater photometer with suspensions of soft sediment until no light was measured. Even on the brightest days, no light penetrated more than 0.3 cm of sediment.

Sedimentary chlorophyll decreased to a constant level with depth in the sediment and also declined with increasing depth of water over the sediment (Fig. 2) .These changes correspond to changes in algal biomass (G. Greundling, personal communication). Thus, although the technique for sedimentary chlorophyll measures total chlorophyll in various stages of decomposition,

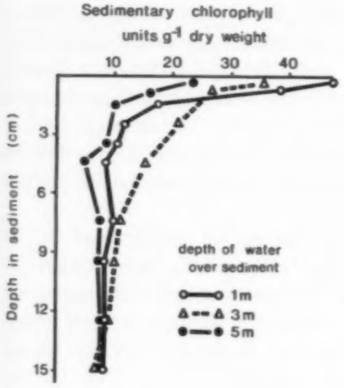

FIG. 2. Sedimentary chlorophyll, units g^{-1} dry weight, in sediment covered by various depths of water in Marion Lake, B.C.

these analyses may provide a useful index of epibenthic algal standing crop. High concentrations of pigments at the surface, also noted by Pomeroy (1959), would seem to indicate the limit of algal penetration into the sediment.

To calculate annual gross oxygen production by epibenthic algae, weekly averages of light, temperature, and day length (Fig. 3) and values calculated for community respiration (discussed below) were substituted into the multiple regression derived for all stations (Table 1). Calculations were made for sediment covered with 0.5 m of water and successive 1.0-m depth intervals. Since light extinction varied with season (Fig. 4), radiation incident at the sediment at each depth was calculated accordingly. The values thus derived, as milliliters O_2 m^{-2} hr^{-1} produced on a weekly average, were multiplied by day length to determine average daily production throughout the year. Annual production of oxygen at each depth was measured as the area under the curve of weekly averages. Figure 5 illustrates these calculations for 0.5, 2.5, and 4.5 m depths and annual gross oxygen production at all depths is summarized in Tables 4 and 5.

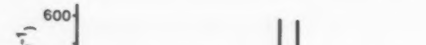

FIG. 3. Weekly mean light incident at lake surface, day length, and temperature during 1968 in Marion Lake, B.C.

Gross oxygen production was converted to carbon by a factor of 0.536 (Strickland, 1960). Algal weight, assumed to be 50% carbon, was converted to calories on the basis of 4.9 Kcal g^{-1} (Cummins, 1967) which was compared with photosynthetic radiation reaching the sediment surface to calculate photosynthetic efficiency at each depth (Fig. 6). Pomeroy (1959) found similar efficiencies for algae on a Georgia salt marsh.

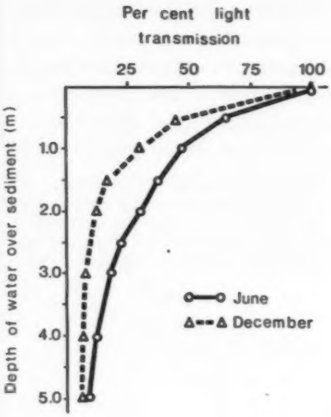

FIG. 4. Light extinction with depth in Marion Lake during June and December 1968. Curves used to calculate the amount of incident radiation reaching sediment covered by different depths of water.

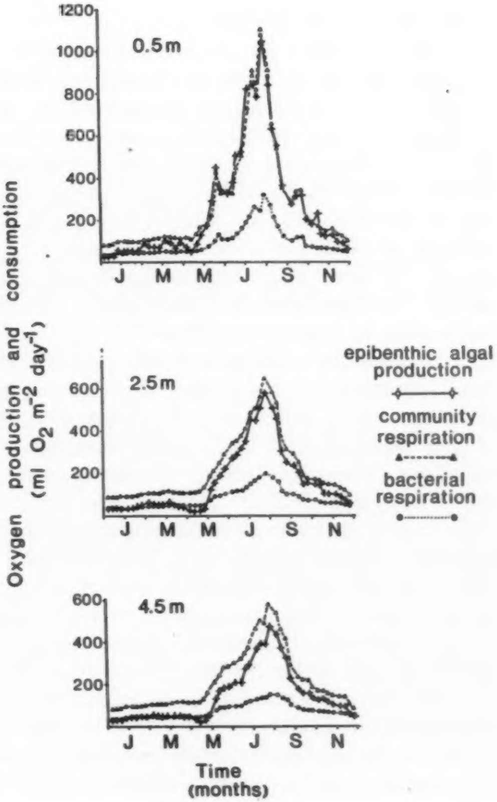

FIG. 5. Summary of weekly average values calculated from regression equations describing gross oxygen production, total community respiration, and bacterial respiration. The area under the curve was used to estimate total annual production and respiration in sediment covered by various depths of water in Marion Lake, B.C.

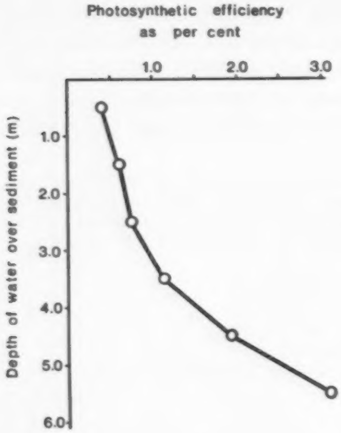

FIG. 6. Gross epibenthic algal production, converted to calorific equivalents, compared with incident photosynthetic radiation at sediment surface to estimate photosynthetic efficiency of epibenthic algae on sediment at various depths in Marion Lake.

COMMUNITY RESPIRATION

Oxygen uptake by undisturbed sediments, corrected for chemical uptake, may be used as an index of community heterotrophic activity. This can be compared with autotrophic production to evaluate the relative importance of benthic primary production as an energy source for the benthic community. A multiple regression analyses was performed, as described for oxygen production, which compared oxygen uptake with temperature, oxygen concentration, time of day, day length, and depth of water over the sediment. The analysis showed a significant regression on all variables except depth. The addition of time^2 as a variable significantly improved the fit of the regression, as did transformation of temperature, oxygen concentration, and sediment respiration to logarithms (Table 1).

Temperature affects both the rate of oxygen diffusion across the mud–water interface and respiration of organisms in the sediment. The importance of temperature, which is interrelated with oxygen concentration, in determining respiration of benthic communities from different areas is discussed elsewhere (Hargrave, 1969a). To summarize these results, the similarity of the relationship between temperature and respiration in different sediment communities suggests that oxygen availability is an important factor in influencing oxygen consumption by sediments. This is apparent in Marion Lake from the significant effect of oxygen concentration on community respiration (Table 1) and from experiments which have shown sediment respiration to be a function of dissolved oxygen concentration below 6.0 ml O_2 liter^{-1} (Edwards and Rolley, 1965; Hargrave, 1969a).

Short-term incubation of sediment cores usually prevented oxygen concentrations from falling below 6.0 ml O_2 liter^{-1} during respiration experiments in Marion Lake. Correction for reduced oxygen concentrations would only be necessary during the summer when increased temperatures and higher respiration rates reduced oxygen level slightly below 6.0 ml O_2 liter^{-1}. At other times of the year water over sediments in Marion Lake was well-aerated with a dissolved oxygen concentration which approached saturation (Fig. 7). Thus, respiration rates calculated during July and August, when oxygen concentration was minimal, may be slight underestimates of community oxygen consumption which occurs under natural conditions.

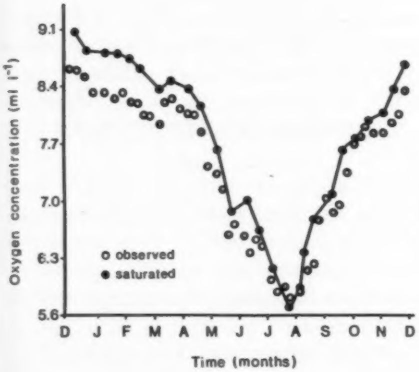

FIG. 7. Dissolved oxygen concentration in water over sediment in Marion Lake during 1968 (*open circles*). Saturation concentrations of dissolved oxygen (*solid circles*) calculated for in situ temperatures during the year.

The significant effect of time of day on sediment respiration in Marion Lake (Table 1) was apparent in preliminary experiments with sediment cores incubated at various times during the day and night (Fig. 8). Respiration rates of sediment cores incubated at night were two or three times higher than similar cores incubated during the day. Odum (1957) found similar results and suggested that oxygen produced by epibenthic algae during the day diffused into overlying water during short-term respiration experiments, thus reducing the apparent respiration rate. Bubble formation was observed

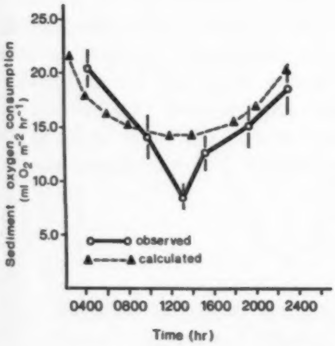

FIG. 8. Respiration of sediment cores from Marion Lake incubated at various times of day (*open circles*). Temperature (16 C) and dissolved oxygen concentration of water over sediment (6.5 ml O_2 liter^{-1}) did not vary during the day. Mean and standard error of three cores incubated at each time interval shown. Dotted line indicates estimates of sediment respiration derived from regression equation (Table 1) at various times of the day.

to occur in the algal mat layer on sediments in Marion Lake during sunny days. Attempts to measure changes in oxygen concentration in interstitial water during the day were unsuccessful, but the oxygen free to diffuse from sediment into overlying water should be a function of the rate of photosynthesis prior to respiration experiments. This is suggested in my data, since on several sunny days, with maximum algal production, minimum respiration at midday was significantly lower (8.0 ± 2.0 ml O_2 m^{-2} hr^{-1}) than similar measurements on cloudy days (14.5 ± 2.5 ml O_2 m^{-2} hr^{-1}) ($P < 0.05$).

Additionally, actively photosynthesizing algae could continue to produce oxygen for a short time after removal from the light. Either of these processes would reduce changes in dissolved oxygen concentration over sediment cores and community respiration would appear to decrease. In either instance, the most realistic measure of community oxygen uptake would be in experiments conducted at night.

An alternative explanation for diel changes in sediment oxygen consumption involves an endogenous metabolic rhythm, as found in laboratory microecosystems by Beyers (1963). *Hyalella azteca*, one of two amphipod species found in the sediment in Marion Lake, has been used in laboratory experiments (Hargrave, 1969b). No diel changes have been observed in feeding or respiratory rates of these animals.

Day length was positively correlated with community respiration. This probably reflects increased biomass of the benthic community during the summer months. Even with this parameter, however, only 76% of the variation in community respiration was accounted for by the regression in Table 1 (MR^2 values of 0.76). Spatial differences in oxygen uptake were large (±20%) in comparison with those observed for gross oxygen production. Some of this variation might result from nonhomogeneous distribution of invertebrates and microfauna and flora within the benthic community.

There was no significant difference in oxygen consumption at different stations once the variation due to parameters listed in Table 1 was considered. Thus, all data were pooled and annual sediment community respiration was calculated from the multiple regression in a manner described for epibenthic algal production. Mean weekly temperatures and oxygen concentrations (Fig. 7) were substituted. No correction for time of day has been made since the omission of this parameter maximizes respiration. The calculated effects of this variable, however, are presented in Fig. 8.

PARTITIONING COMMUNITY RESPIRATION

BACTERIAL RESPIRATION

Oxygen uptake by bacteria in the sediment was measured at least once per month at each station. A multiple linear regression showed that respiration was related only to temperature, with no significant effect of oxygen concentration, day length, depth, or time of day. Logarithmic transformation of bacterial respiration significantly improved the regression and data from all stations were combined on this basis (Fig. 9). Annual bacterial respiration

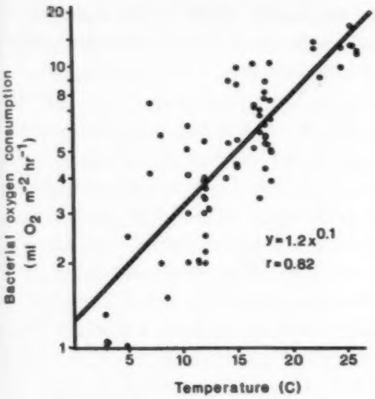

FIG. 9. Relationship of temperature and sediment bacterial respiration in Marion Lake sediments during 1968.

was calculated as described for algal production and community respiration (Fig. 5).

Only 68% of the variation in bacterial respiration was accounted for by the regression on temperature ($R^2 = 0.68$). Spatial heterogeneity (±20%), as measured in replicate cores from one sampling site, was greater than spatial variation found in oxygen production. Variation in bacterial flora on sediment surfaces only a few centimeters apart, as found by Lackey (1961), could account for such differences.

Bacterial respiration was independent of oxygen concentration within the range experienced during this study (5.7–8.6 ml O_2 $liter^{-1}$). ZoBell and Stadler (1940) reported similar results. This is not surprising, perhaps, since most sediment microorganisms are facultative aerobic and anaerobic species which may adjust their respiration rate to correspond to the oxygen available.

The proportion of community respiration due to bacteria varied with season (Fig. 10). During the summer, when total community metabolism increased owing to higher temperatures, bacteria accounted for less than 30% of the oxygen consumed by the community. This proportion increased to a maximum in December when 45% of community respiration was inhibited

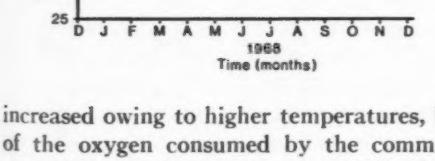

FIG. 10. Bacterial respiration expressed as a proportion of total community respiration in Marion Lake sediments during 1968.

by antibiotics. These results contrast those of Carey (1967) and Kanwisher (1962) who estimated that bacterial respiration in marine sediment cores accounted for over 70% of the oxygen consumption.

OXYGEN UPTAKE BY BENTHIC FAUNA AND FLORA

The relative importance of various organisms in the metabolism of a benthic community can be determined by comparing their respiration rates. Also, algal respiration can be estimated by difference when total community respiration is corrected for oxygen consumption by heterotrophic organisms. To make such comparisons, oxygen consumption of macrofauna separated from sediment was measured (Table 2). Respiration rates are means of at least three determinations with average sized animals collected during June 1968. Densities are mean summer values observed by Hamilton (MS, 1965) supplemented by counts made during 1968 (B.T. Hargrave, unpublished data).

TABLE 2. Partitioning community respiration in sediments of Marion Lake. Total community respiration, June 1968, 16.0 ml O_2 m^{-2} hr^{-1}, 15 C; *y* = young, *a* = adults. Numbers in italics indicate estimates by calculation.

	No. per m^2	μliters O_2/animal per hr	μliters O_2 m^{-2} hr^{-1}	Dry wt per individual (*mg*)	Total dry wt (*mg/m²*)
Bacteria	*1.2×10^{11}*	4×10^{-8}	5.0	10^{-10}	12
Algal Microflora	*2.8×10^{9}*	1.3×10^{-6}	*3.7*	10^{-8}	28
Protozoa	2.0×10^{6}	1.0×10^{-3}	2.0	10^{-4}	200
Amphipoda					
Hyalella azteca	1500 (*y*)	0.4	0.6	0.15	225
	800 (*a*)	0.8	0.6	0.80	640
Crangonyx	100 (*y*)	0.6	0.06	0.4	40
richmondensis	50 (*a*)	1.0	0.05	1.2	60
Chironomidae					
Tanypodinae	1000	0.4	0.4	0.25	250
Orthocladinae	600	0.5	0.3	0.25	150
Chironominae	3000	0.5	1.5	0.50	1500
Copepoda	3000	0.1	0.3	0.01	30
Mollusca					
Pisidium	600	0.5	0.3	1.25	750
Menetus	50	3.0	0.2	10.0	500
Oligochaeta					
Lumbriculidae	100	1.0	0.1	1.3	130
Tubificidae	800	0.5	0.4	1.0	800
Hirudinea					
Glossiphoniidae	50	3.0	0.1	2.6	130
Trichoptera					
Phryganeidae	30	10.0	0.3	15.0	450
Odonata					
Zygoptera	5	5.0	0.03	1.3	7
Anisoptera	3	30.0	0.03	90.0	270
					6172 mg

Dry weight determinations (Table 2) were compared with measures of oxygen uptake for all macrofauna species in the community (Fig. 11). Logarithmic transformation of both axes makes the curve linear and comparable with similar data presented by Zeuthen (1953). The slope constant is not significantly different ($P < 0.05$) from that derived by Zeuthen. Oxygen uptake by protozoa and rotifers was calculated using this regression and an average cell dry weight of 10^{-4} mg. Zeuthen (1953) cited fresh weight values ranging from 10^{-5} mg for ciliates to 10^{-2} mg for large amoeba. Data on average densities of protozoa and rotifers in shallow-water sediment in Marion Lake were supplied by Miss Dianne Smith (personal communication).

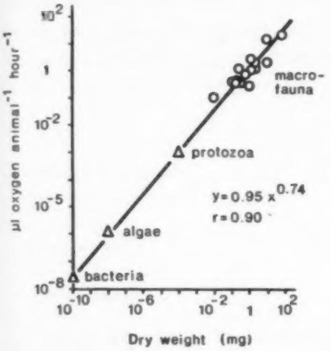

FIG. 11. Relationship between dry weight and oxygen consumption at 15 C macrofauna separated from sediment (*circles*). Triangles represent extrapolated values used to calculate protozoan, algal, and bacterial respiration rates. Regression equation and correlation coefficient (r) ($P < 0.05$) as shown in figure.

Oxygen uptake by algal microflora was calculated as 3.7 ml O_2 m^{-2} hr^{-1} (total community respiration corrected for the sum of macrofauna, bacterial, and protozoan respiration). Gross oxygen production by epibenthic algae at 15 C under in situ light conditions during June 1968 was 25.0 ml O_2 m^{-2} hr^{-1}. Thus, net algal production during June was approximately 85% of gross photosynthesis. Pomeroy (1959) estimated that net production in algal populations on a Georgia salt marsh was not less than 90% of gross production. Odum (1959), on the other hand, presented data which showed that plants respire an average of 30% of their gross production. Such variation probably reflects species differences and the effect of physiological state on respiration and they illustrate the difficulty of extrapolating from one community to another.

Respiration rates of individual algal and bacterial cells can also be obtained from the regression of weight on oxygen uptake, using average cell weights taken from Zeuthen (1953). Since respiration per m^2 by bacteria and algae were estimated, cell numbers were calculated as 1×10^{11} and 3×10^9 per m^2 respectively. On several occasions during the summer of 1968 the technique of Jones et al. (1948) was used for direct cell counts on sediment particles. By this method there were 10^8-10^9 bacteria and 10^5 algal cells per gram wet weight of sediment (taken as equivelent to 1 cm^2). These densities include both active and nonactively respiring cells; however, they are comparable with those order-of-magnitude estimates derived in Table 2. The bacterial numbers also approximate those observed in some eastern Canadian lakes by Hayes and Anthony (1959).

Community dry weight, obtained by summing combined numbers and dry weights for each group, was calculated as 6.2 g m^{-2} (Table 2). Sediment bacteria, microflora, and protozoa accounted for 67% of community respiration, but represented only 4% of the dry weight, when oxygen uptake and total dry weight were compared on a percentage basis (Fig. 12). Macrofauna, on the other hand, respired 33% of the oxygen consumed and represented 96% of community biomass during June. Chironomids of the family Chironomini were the single most important faunal group, accounting for 9% of the respiration and comprising 24% of the community weight.

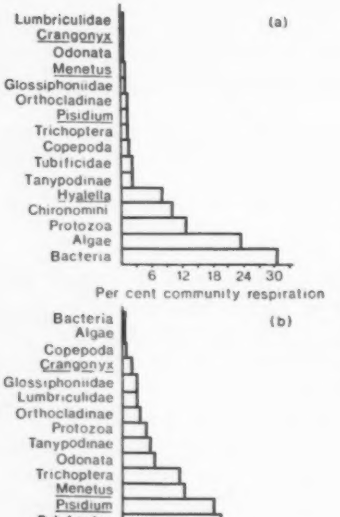

FIG. 12. (*a*) Partitioning of community respiration between various members of the benthic community in Marion Lake during June 1968. In situ total community respiration 16.0 ml O_2 m^{-2} hr^{-1}. Temperature 15 C. (*b*) Partitioning of community dry weight between various members of the benthic community in Marion Lake during June 1968. Total dry weight 6.2 g m^{-2}. *Hyalella* (Amphipoda), *Crangonyx* (Amphipoda), *Pisidium* (Mollusca, Pelecypoda), *Menetus* (Mollusca, Gastropoda).

No attempt has been made to consider the effect of seasonal changes in animal abundance on the proportion of respiration attributable to various organisms. Hamilton (MS, 1965) and Mathias (MS, 1967) recorded summer maxima in numbers of chironomids and amphipods in Marion Lake; however, peak biomass is reached during late fall (B. T. Hargrave, unpublished data). Increases in algal standing crop probably occur during the summer also. Such changes will markedly affect the contribution of each group to total community respiration. The proportionate decrease in bacterial respiration to total community oxygen consumption during the summer (Fig. 10) may reflect these changes. Other workers, however, have compared macrofauna respiration and total community oxygen uptake during the summer when macrofauna respiration would be expected to be maximal (Table 3). Although different types of benthic communities are involved, combined with the present study, these

TABLE 3. Comparison of total community respiration and estimates of oxygen consumption by macrofauna in various benthic communities.

Location and source		Total sediment respiration (*ml* O_2 *m*$^{-2}$ *hr*$^{-1}$)	Estimated macrofauna respiration (*ml* O_2 *m*$^{-2}$ *hr*$^{-1}$)	Macrofauna respiration as % of total
Spartina salt marsh, Wood's Hole (Wieser and Kanwisher, 1961)		26–36	11–16	35–48
River mud (Edwards and Rolley, 1965)		89	35	40
Long Island Sound sediment (Carey, 1967)		24	3.5	17
Marion Lake (present study) (June)		16	5.3	33
Intertidal sandflats (Pamatmat, 1968)	Jan.	5.1	1.7	34
	May	46.0	9.4	21
	Sept.	44.6	8.2	18
	Mar.	3.7	0.4	10
	June	16.0	1.2	8

limited data show that macrofauna account for less than 50% of total community respiration. By difference, bacteria, microflora, and protozoa would account for the remaining oxygen uptake. This suggests that bacteria are of less importance in oxygen comsumption by benthic communities than is generally assumed (Kanwisher, 1962; Carey, 1967). In Marion Lake, for example, during the summer bacteria and macrofauna each account for one-third of the total oxygen consumed.

CARBON FLUX IN MARION LAKE SEDIMENTS

Attempts have been made to evaluate the relative importance of various energy sources in the benthic community of Marion Lake by comparing processes of carbon addition and removal. Calculations of gross oxygen production by benthic algae were converted to carbon (Tables 4 and 5) and a weighted mean for the effect of depth on gross benthic algal production was derived.

TABLE 4. Annual gross oxygen production and consumption (liters O_2 m^{-2} $year^{-1}$) by the benthic community at various depths in Marion Lake during 1968. Values are calculated from the area under the curve of weekly averages of oxygen production and consumption as estimated from regression equations (Table 1). $\bar{x}$ is the weighted mean on the basis of the area of lake bottom at each depth.

Depth (*m*):	0–1	1–2	2–3	3–4	4–5	5–6	$\bar{x}$
% of lake bottom at each depth	41.5	28.1	13.6	10.3	5.1	1.9	
Gross oxygen production	89.4	77.4	57.8	52.5	50.4	35.0	75.4
Community respiration	97.1	93.4	79.2	75.5	74.5	66.8	90.2
Bacterial respiration	32.5	31.5	29.5	27.5	26.0	24.5	31.0

This was combined with measures of phytoplankton and macrophyte production to estimate total annual carbon addition to the sediment by autotrophic processes (Fig. 13). Primary production by phytoplankton (Efford, 1967) and macrophytes (Davies, MS, 1968) in Marion Lake have been measured as 8 and 18 g C m^{-2} $year^{-1}$ respectively. Since C^{14} techniques were used in both studies, something less than gross primary production was measured and the values are not directly comparable with those derived during this study.

TABLE 5. Weighted means of oxygen production and consumption (liters O_2 m^{-2} $year^{-1}$) expressed as g C, g O_2 and Kcal m^{-2} $year^{-1}$. A photosynthetic quotient of 1.0 and a respiratory quotient of 0.85 are assumed. Dry weight of organisms assumed to be 50% carbon with a calorific content of 4.9 Kcal g^{-1}.

	Liters O_2 m^{-2} $year^{-1}$	g C m^{-2} $year^{-1}$	g O_2 m^{-2} $year^{-1}$	Kcal m^{-2} $year^{-1}$
Gross epibenthic algal production	75.4	40.4	107.8	395.9
Community respiration	90.2	56.9	129.0	402.8
Bacterial respiration	31.0	19.5	44.3	138.2

Also, whereas most macrophyte production probably remains in the lake, Dickman (1969) showed that phytoplankton production and standing crop in Marion Lake are reduced by high flushing rates. Thus, it is assumed that all macrophyte production reaches the sediment, whereas only 10% of the carbon fixed by phytoplankton is added annually.

Allochthonous organic debris from the inlet stream of Marion Lake may also add carbon to the sediment. Suspended particulate matter in the inlet

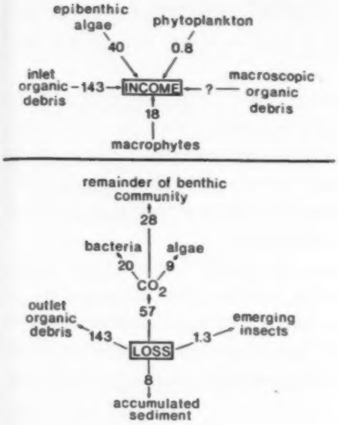
epibenthic algae
40
phytoplankton
0.8
inlet organic debris
143
INCOME
?
macroscopic organic debris
18
macrophytes
remainder of benthic community
28
bacteria
20
algae
9
CO_2
57
outlet organic debris
143
LOSS
1.3
emerging insects
8
accumulated sediment

FIG. 13. Mean annual carbon flux (g C m^{-2}) in sediments of Marion Lake. All measurements are empirically derived. Values represent weighted means for the effect of depth.

and outlet streams was measured during 1966 (I. E. Efford, unpublished data) and found to be equivalent. Ash-weight determinations showed that 65% of this material was organic. If it is assumed that 40% of this organic material is carbon, then 143 g C m^{-2} $year^{-1}$ is added and removed in the inlet and outlet streams. Although there may be no net addition of particulate matter from this source, this addition represents a major supply of sediment carbon. No measure of coarse particulate matter (leaves and other macroscopic terrigenous material) has been made.

Carbon may be removed from the sediment as carbon dioxide produced during respiration by the benthic community. Such losses (Tables 4 and 5) were calculated from measurements of oxygen uptake by undisturbed sediment by assuming a respiratory quotient of 0.85. Since bacterial and algal respiration rates have been calculated, annual respiration of carbon dioxide has been divided accordingly.

The release of soluble organic substances has not been measured, but concentrations of free reducing sugars at the mud–water interface and in interstitial water were low (10^{-6}–10^{-7} M) (B. T. Hargrave, unpublished data). This may indicate rapid recycling of such compounds within the sediment with little net loss. No measurement of dissolved organic compounds in inlet and outlet streams have been made on an annual basis.

Loss of sediment through turbulence and water transport is included in measures of particulate matter in the outlet stream. Emergence of larval insects (Chironomidae, Odonata, and Trichoptera) represent a loss of sediment carbon. A maximum value of 1.3 g C m^{-2} $year^{-1}$ can be calculated if the density of emerging adults is assumed to be equivalent to the mean summer density of larvae and nymphs with a carbon content of 40% of the dry weight (Table 2).

Sediment carbon may also be removed from a benthic community by burial below the aerobic surface sediment layer. Sediment in Marion Lake has accumulated at 0.5 mm per year (Dr G. Rouse, personal communication).

Subsurface sediment is 90% water and 40% of the dry weight is organic (by loss of weight at 600 C for 1 hr). If this material is 40% carbon, then 8 g C m^{-2} $year^{-1}$ accumulates.

Summation of carbon lost annually totals 209 g C m^{-2} (Fig. 13). Particulate removal forms 69% of this loss, and respiration accounts for an additional 27%. Only 4% of the carbon added to the sediment annually remains to accumulate. This illustrates the dynamic nature of the benthic community in Marion Lake, whereby 96% of the annual carbon supply is either metabolized by the community or removed by physical transport.

No estimates of community efficiency can be made from these data, since the pathways and rates of carbon transfer within the sediments are not known. The nature of organic material in inlet and outlet streams, the organic substrates utilized by sediment bacterial populations and the amount of epibenthic algal production consumed within the community must be known before such calculations can be made.

CONCLUSIONS

Oxygen changes over undisturbed cores offer a rapid, sensitive, and reproducible method for the direct evaluation of autotrophic and heterotrophic processes within benthic communities, provided possible chemical uptake is considered. In Marion Lake these techniques have shown that 71% of the carbon respired by the benthic community annually could be supplied by epibenthic algal production (Fig. 13). The actual amount of benthic primary production respired by the community is unknown, since allochthonous organic material from the inlet stream and sides of the lake may also be metabolized. Since, however, benthic algae respired only 15% of the total carbon fixed, this primary production is probably an important energy source for the benthic community.

The importance of epibenthic algal production to total photosynthetic carbon fixation in shallow lakes, ponds, rivers, and marshes has been stressed by Wetzel (1964). Comparison of estimates of primary production in various aquatic ecosystems has been made to further emphasize the significance of this production (Table 6). A review of plant productivity by Westlake (1963) summarizes much of the data available on phytoplankton production. It is apparent that in shallow-water ecosystems, primary production on sediments exceeds that occurring in the water column.

Sedimentary chlorophyll analyses indicate a high algal standing crop at the sediment surface. Such stratification has often been observed (Taylor and Gebelein, 1966; Moss, 1968) and is generally related to the depth of light penetration into the sediment. In Marion Lake, as in other aerobic benthic communities, oxygen and macrofauna show similar vertical distributions (Jansson, 1967; Fenchel and Jansson, 1966; Milbrink, 1968). Such observations confirm the existence of Naumann's (1930) "actual layer" where biological activity occurs in surface sediment, and an "historical layer" of highly reduced, anoxic, subsurface sediment.

TABLE 6. A comparison of primary production by different communities of various aquatic ecosystems.

Type of community	Production (*g C* m^{-2} *year*$^{-1}$)	Method of measurement	Reference
Marion Lake			
Epibenthic	40	Oxygen	Present study
Phytoplankton	8	C^{14}	Efford (1967)
Macrophytes	18	C^{14}	Davies (MS, 1968)
Borax Lake			
Periphyton	267	C^{14}	Wetzel (1964)
Phytoplankton	91	C^{14}	
Macrophytes	28	C^{14}	
Ocean phytoplankton	80		Westlake (1963) review of published data on plant productivity
Lake phytoplankton	80		
Coastal phytoplankton	120		
Submerged macrophytes	240		
Epibenthic algae, Georgia salt marsh	200	Oxygen	Pomeroy (1959)
Epibenthic intertidal sandflat	143–226	Oxygen	Pamatmat (1968)
Intertidal marine sediments	115–178[a]	C^{14}	Grøntved (1962)

[a] Recalculated by Pamatmat (1968).

Moss (1968) also observed that algal standing crops on sediment surfaces may have a chlorophyll *a* content comparable with phytoplankton on an area basis. Benthic algae, however, are concentrated within the upper few centimeters of sediment, rather than distributed through the water column. Thus, on a volume basis, chlorophyll concentrations in sediment are much higher than those found in the water column. This may account, in part, for the relatively high photosynthetic efficiencies (0.4–3.1%) found for benthic algae during this study and by other workers (Pomeroy, 1959; Pamatmat, 1968). Photosynthetic efficiencies of natural phytoplankton populations are generally below this level. Efford (1967) showed that phytoplankton in Marion Lake converted photosynthetic energy to plant material with a 0.09% efficiency (recalculated for an arithmetic error) which compares well with data from other planktonic algal communities (Vinberg, 1960). The stability of benthic communities and nutrient availability may also contribute to higher photosynthetic efficiencies.

Temperature was the most important variable affecting oxygen production and consumption by the benthic community in Marion Lake. Over 70% of the variation in community respiration could be accounted for by this variable. It must be recognized, however, that in correcting for temperature many other related variables which are temperature-dependent are also being considered. In any case, sediment organic content and species composition appear to have a minor effect on oxygen consumption in many natural benthic communities (Hargrave, 1969a). Processes of oxygen supply and demand in undisturbed aerobic benthic communities may be balanced and related to temperature through processes of diffusion. Thus, at any given temperature and oxygen concentration, only a certain amount of oxygen is available to the community and this is fully utilized.

Although few studies exist for comparison, the similarity of respiration in different benthic communities may extend to the relatively similar proportion of community respiration due to macrofauna. The relation between biomass and metabolic rate offers a rapid method for determining the respiratory contribution of various members of the benthic community, although problems of enumeration and extrapolation of laboratory-determined respiration rates to nature remain. As more data accumulate, the relative importance of macrofauna, microfauna, and flora and bacteria in benthic community respiration will be better assessed. In this study, however, during June each group respired one-third of the total oxygen consumed.

While this manuscript was in preparation, a study of oxygen production and consumption in an intertidal sand flat was reported (Pamatmat, 1968). A summary of Pamatmat's extensive observations on community respiration and epibenthic algal production are included in Tables 3 and 5. Estimates of macrofauna respiration were carried out in different months and these results are summarized in Table 3. Although bacterial respiration was not measured directly, Pamatmat's estimates of oxygen consumption by microfauna plus bacteria are comparable with similar measurements in the present study. These results indicate similarities in metabolic processes in benthic communities which are independent of differences in species composition. Further studies will be required to quantify these similarities and determine their cause.

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Newfoundland Capelin: Proximate Composition[1, 2]

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Proximate analysis data for whole beach-spawning capelin from various parts of Newfoundland collected over a period of 20 years is generally similar when allowance is made for trends occurring during the brief spawning season. These trends, more evident in males, are, concurrently, a sharp decline in fat content, a slight increase in moisture, and a small decrease in protein. Ranges observed for these variables on a wet weight basis were, respectively, 8.1–1.8%, 77.1–82.3% and 15–12.9% (crude protein). Similar values were obtained for muscle tissue.

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INTRODUCTION

The capelin (*Mallotus villosus*), with its spectacular spawning migration to the beaches of Newfoundland, Labrador, and parts of the estuary of the St. Lawrence river, continues to form a tantalizingly undeveloped fishery. It is probable that the resource available may be measurable in hundreds of millions of pounds, of which some 10 million lb are at present harvested in Newfoundland for bait and other local use (Templeman, 1966). A conference on the potential of this species was organized by the Department of Industry and held in Ottawa in the Department of Fisheries Conference Room, February 1, 1967.[5] With the decline in abundance, due to fishing pressures, of large fish such as cod (*Gadus morhua*) which consume quantities of capelin, there may be an increase in the stock of capelin available for direct exploitation (W. Templeman, Ottawa Conference, 1967).

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[1] The 16th paper on Newfoundland fish.

[2] This project was supported in part by funds from the Industrial Development Service of the Department of Fisheries of Canada.

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[5] The proceedings of this conference will not be published.

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There has been a considerable expansion in fishmeal plant capacity in Newfoundland in recent years (Jangaard, 1967), primarily for reduction of herring (*Clupea harengus*). The new plant capacity and the availability of capelin inshore, and offshore prior to spawning, may lead to exploitation for meal and oil on a much larger scale than at present as echo-location, purse seining or midwater trawling, and reduction technology can be adapted to this species (F. N. Clarke, Ottawa Conference, 1967). The present tabulation of data on the proximate composition of the capelin is intended as a guide to the properties that may be expected for capelin taken in Newfoundland, but is based with one exception on beach-spawning samples. Should an offshore fishery develop it might include fish that spawn in deeper water and therefore are likely to be comparable with inshore spawners, or it might include schools of maturing capelin with much higher fat contents (Winters, 1968) and a better "condition" which facilitates reduction (F. N. Clarke, P. M. Jangaard, Ottawa Conference, 1967).

Details of fat and fatty acid composition, of seasonal trends in fat composition, and of alterations in fat during frozen storage of whole fish, including some other 1966 samples, are presented elsewhere (Ackman et al., 1969).

SAMPLES AND METHODS

The samples analyzed in 1951 and 1953 are summarized from the original report (Andrews, 1954) and details on analytical procedures are not available beyond the notation that Bailey-Walker fat extraction was employed. The seven samples of whole fish came from only two lots of fish, described as fresh.

The 1960 samples were fresh (still in rigor) when examined in Halifax, N.S. The fish were headed, gutted, skinned, and filleted. Sample data therefore refer to *flesh only*. Analytical procedures have been described by Mannan et al. (1961a); moisture was determined by drying to constant weight at 105 C, ash at 550 C, lipid by the chloroform–methanol procedure of Bligh and Dyer (1959), total nitrogen by Kjeldahl digestion and nonprotein nitrogen on 5% trichloroacetic acid extracts by a microkjeldahl procedure.

Essentially the same procedures were used for analyses of other samples (whole fish). Salt was determined as described by Dyer (1943). Free fatty acids were determined by direct titration of lipid extract (Jacobs, 1959). No correction was made for acidity of the phospholipids (cf. Lovern, 1965; Rhodes and Lea, 1961). The samples, with the exception of four from Middle Cove where only the per cent lipid values are given, were analyzed from 1 to 3 weeks after capture and immediate freezing. In these four exceptions, lipid was determined after several months of frozen storage; some drying out as well as some increase in free fatty acid may have occurred in these samples but little change should have occurred in the other samples analyzed.

RESULTS AND DISCUSSION

In comparing fat contents (Table 1) note that samples in 1951 and 1953 were extracted by conventional techniques using ether, and samples in 1962 were extracted with benzene. All other work since 1960 has been carried out with the efficient chloroform–methanol system of Bligh and Dyer (1959). This may give results as much as 30% higher than ether extraction when fat is very low ($<3\%$), but the differences are less important as the fat levels rise.

The fillet flesh, which was analyzed from only one lot of beach-spawning capelin (1960, Table 1), differs in composition from that of most teleost fish

(Fraser et al., 1961) especially in its lower protein content. In this it compares with the Atlantic sea sturgeon (*Acipenser oxyrhynchus*) and it also has similar moisture and fat contents (Fraser et al., 1961). The crude protein, about 15% (Table 1) is markedly lower than in the mackerel, tuna, and swordfish groups (about 20–24%) as is also nonprotein nitrogen, whereas moisture is slightly higher (Mannan et al., 1961b). Although it has been suggested that the capelin may be related to the Salmonidae (see Ackman et al., 1969), its protein is much lower than in the flesh of several salmon species which average 20–22% protein: *Salmo salar* (Taarland et al., 1958), *Oncorhynchus gorbuscha* (Thurston and Groninger, 1959), and *Oncorhynchus nerka* (Idler and Bitners, 1958). The sum of moisture plus fat in these salmon species varies from 77 to 80% (77.5–82% in most teleosts; Fraser et al., 1961) whereas that of the capelin flesh sample is 84.4% (male + female) as is also that of the Atlantic sea sturgeon. The protein content of herring flesh (*Clupea harengus*) varies considerably, from 18% and 17% reported by Clark and Almy (1918) and by Taarland et al. (1958) to values of 15.1–16.5% for spawned Scottish herring, whereas in herring spawned and starved (in addition) it was as low as 14.6% (Wilkins, 1967). Thus, in protein content the capelin is closer to the herring than to the salmon group, though the low protein level may be partly due to the spawning condition as in the herring.

In the whole fish average crude protein content is lower than in the fillet flesh (14% vs. 15%) presumably because of the admixture of bone and collagen in the former. Nonprotein nitrogen on the other hand is considerably higher (0.42%) as compared with 0.33% in the flesh, so that the true protein values differ even more than the crude values, 11.2% as compared with 12.9% respectively. Unfortunately, no data were obtained on the March offshore trawled fish and the differences in the beach-spawning lots are small. However, as the spawning season progresses from mid to late June, there is a tendency, more evident in the male fish, for an increase in moisture (Bonavista Bay and Fortune Bay lots) with accompanying loss of fat but with little decrease in protein (approximately 7% in the Fortune Bay male fish).

No difference appears in the overall average values between male and female fish in the lots reported in Table 1 in either average moisture (80.3 and 79.3%, crude protein (13.7 and 14.2%), or nonprotein nitrogen (0.43 and 0.42%).

In the late June (one early July) beach-caught fish, no significant difference appears in average fat content between male (15 lots) and female (14 lots) fish (4.14 and 4.26% respectively with standard deviation of ±2.0 and ±1.0). In some lots the females are considerably more fatty and in others the reverse relation holds. In the one lot trawled offshore in Trinity Bay on March 5, 1967, the fat content of the female fish (8.9%) was much higher than that of the males (3.2%). Samples from the same lot reported by Winters (1968) showed no difference between sexes. However, the level of fat (12.8%) was considerably higher than in the present samples and this level, as well as the ratio of fat level between the sexes, may be related to the size of the fish.

TABLE 1. Origin, nature, and chemical analysis data for samples of Newfoundland capelin as per cent of wet weight (except free fatty acid which is expressed as per cent of lipid using a mol wt of 300). Data on the Conception Bay samples of 1951 and 1953 are from Andrews (1954).

	Sample data												
Location of catches[a]	Date	No. fish	Sex	Avg length (*cm*)	Moisture	Lipid	Free fatty acid (in lipid)	Crude protein (as N × 6.25)	% of non-protein nitrogen	True protein	Ash	Salt	Remarks
					Conception	**Bay**							
Lance Cove (Bell Is.)	June 20, 1951	10	M	18.5	80.73	3.23^b	–	13.64	–	–	–	–	Partly spent
	"	10	M	18.3	79.98	4.12^b	–	13.60	–	–	–	–	Partly spent
	"	10	F	16.2	79.55	3.37^b	–	13.83	–	–	–	–	Full of eggs
Topsail	June 24, 1953	20	M	18.0	80.27	3.64^b	–	14.19	–	–	2.20	–	Partly spent
	"	20	M	17.8	79.93	3.90^b	–	14.19	–	–	2.09	–	Partly spent
	"	20	F	16.0	80.10	3.09^b	–	14.49	–	–	1.84	–	Mixed states
	"	20	F	16.2	80.35	3.06^b	–	14.56	–	–	1.84	–	Mixed states
					Atlantic	**Ocean**							
Torbay (near St. John's)	June 22, 1960	30	M+F	15–18	81.8	2.6	–	14.96	0.33	12.87	–	–	Partly spent
	"	12	M	15–18	80.8	3.8	–	–	–	–	–	–	*Flesh only*
	"	12	F	15–18	84.1	2.0	–	–	–	–	–	–	
	June 27, 1962	52	M	17.9	–	3.35^c	–	–	–	–	–	–	–
	June 27, 1962	56	F	15.8	–	3.39^c	–	–	–	–	–	–	Full of eggs
Middle Cove (near St. John's)	June 22, 1966	–	M	–	80.24	5.69	4.38	13.06	0.39	10.63	–	0.36	–
	June 22, 1966	–	F	–	80.25	5.35	6.01	14.31	0.35	12.13	–	0.24	–
	June 24, 1966	6	M	18	–	1.90	–	–	–	–	–	–	Partly spent
	June 24, 1966	6	F	15	–	3.88	–	–	–	–	–	–	Full of eggs
	June ?, 1966	–	M	–	80.56	7.34	4.40	12.88	0.39	10.44	–	0.11	–
	"	–	F	–	79.36	6.42	7.38	12.94	0.41	10.38	–	0.19	–
	"	15	M	15	–	3.16	–	–	–	–	–	–	Partly spent
	"	9	M	15	–	2.28	–	–	–	–	–	–	Partly spent

Bonavista Bay													
Long Beach, Rolling Cove (between Bonavista and Cape Bonavista)	June 15, 1966	–	M	–	77.13	8.08	5.09	14.13	0.43	11.44	–	0.26	–
	June 15, 1966	–	F	–	78.19	5.11	7.48	14.06	0.41	11.50	–	0.33	–
	June 22, 1966	–	M	–	80.18	7.09	4.65	13.25	0.41	10.69	–	0.43	
	June 22, 1966	–	F	–	78.65	5.47	6.50	13.63	0.42	11.00	–	0.34	–
Freshwater (between Birchy Cove and Bonavista)	June 29, 1966	4	M	–	81.65	2.05	16.13	14.13	0.46	11.25	–	–	–
	June 29, 1966	7	F	–	79.03	5.03	9.05	14.39	0.43	11.69	–	–	–
Trinity Bay													
Lance Cove (or Back-side, near Cape Bonavista)	July 5, 1966	5	M	–	82.26	1.83	17.95	13.06	0.39	10.62	–	–	–
	July 5, 1966	8	F	–	79.90	3.51	10.89	14.61	0.41	12.06	–	–	–
Trawl-caught by *A. T. Cameron*	Mar. 5, 1967	4	M	15	–	3.16	–	–	–	–	–	–	Ripening
	Mar. 5, 1967	4	F	14	–	8.86	–	–	–	–	–	–	Ripening
Fortune Bay													
Kelly's Cove (¾ mile east of Grand Bank Hbr.)	June 16, 1966	8	M	–	79.72	4.32	10.73	14.38	0.49	11.31	–	–	–
	June 16, 1966	6	F	–	79.82	4.59	9.92	14.44	0.45	11.63	–	–	–
100 yards east of Grand Bank Hbr.	June 23, 1966	7	M	–	80.11	3.39	11.58	14.38	0.44	11.63	–	–	–
	June 23, 1966	8	F	–	78.38	3.67	14.36	15.30	0.44	12.56	–	–	–
Boot and Shoe Cove (200 yards east of Kelly's Cove)	June 28, 1966	8	M	–	81.81	2.21	17.77	13.44	0.47	10.50	–	–	–
	June 28, 1966	8	F	–	77.96	3.67	13.32	13.81	0.43	11.13	–	–	–

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[a] Shore-caught except for March 5, 1967, trawl-caught samples from Trinity Bay.
[b] Ether extraction.
[c] Benzene extraction.

Winters' samples averaged 19–20 cm in length compared with 14–15 cm in the present lot (Table 1). The limited size range in the present samplings of beach-caught fish provide little additional evidence on this point. All females were between 15 and 16 cm; the two lots of males 15 cm long were slightly less fatty than the six lots, 18–18.5 cm long, averaging 2.8 and 3.3% respectively. Similar differences appear between five lots of female fish, 15–16 cm long and averaging 2.8% fat, and six lots of male fish, 18–18.5 cm averaging 3.3% fat (Lance Cove, Topsail, Torbay June 27, 1962, and Middle Cove June 24, 1966, June 1966 lots).

The present results show little evidence of interyear variation in levels of fat, but large variations have been reported in the Norwegian capelin fishery (Nitter-Egenaes, 1967). These are apparently related to annual variation in the position of oceanographic cold fronts which affect the energy expenditure necessary for the fish to reach the spawning grounds in any particular year.

There is a tendency, at least in some areas, for the lipid content to decrease over the short spawning season. In the 1966 Fortune Bay lots (three adjacent beaches), it decreases from about 4.5% on June 16 to about 3.5% on June 23. By June 28, though the female remains unchanged, the lipid in the male fish is reduced to 2.2%. The Bonavista Bay lots show no change in the females from June 15 through June 22–29, but in the males there is a decrease from 8.1 to 7.1 to 2.1%. The Middle Cove samples show a drop in fat levels in both male and female lots between June 22 and 24. Accompanying increases in moisture and decreases in nitrogen and protein are much less marked. More extensive sampling would be required to define the extent and significance of the trends.

This apparent decrease of lipid during the spawning period may be partly due to the older and larger fish spawning first, followed by smaller fish which have a different fat level initially or which may lose fat more rapidly while on the beaches (Templeman, 1948; Winters, MS, 1966, 1968). During 1967 (and later, G. H. Winters, personal communication) trawling studies in Trinity Bay indicated that up to the end of March there were mixed schools of mature and immature capelin. These then separated into small schools of mature fish and larger schools of immature fish, with both groups continuing to feed. However, by late May and early June the nonfeeding, older, more mature fish were approaching the beaches in commercial quantities, while just offshore in the bay there were separate schools of smaller but maturing fish, and in the Bay proper, schools of feeding immature fish were present.

Templeman (1948) indicates that whereas male capelin participate in many spawning sessions at the beach and suffer physical injury as a result, the females remain relatively quiescent in schools lying just offshore. At intervals, some females ripen, proceed to the beach, spawn in one session, and thus suffer less injury than the males. This greater expenditure of energy by the males could also account for the relatively greater loss of lipid than that suffered by the females evident in many of the present samples. It must be emphasized, however, that the particular group to which the actual samples of male and female fish belonged is unknown.

The free fatty acid values in the main are higher than would be expected for fresh fish (Mannan et al., 1961b) and probably, though not necessarily, indicate that hydrolysis occurred during, or prior to, the 1–3-week period of frozen storage. Lipid hydrolysis may occur rapidly during slow freezing or at relatively high frozen storage temperatures (Dyer, 1968).

The progressive changes in composition of the capelin during the short spawning season may parallel results in the commercial meal reduction plants. In the early phase of the capelin season it is reported (data from operational experience in one plant that 7 tons of capelin produce 1 ton of meal, whereas later nearly 9 tons of fish are required to produce the ton of meal (F. N. Clarke, Ottawa Conference, 1967). Iles and Wood (1965) and Wilkins (1967) have pointed out that in herring the deposition of fat is partially counterbalanced by a subtle adjustment of the water/solids ratio to maintain desirable biochemical characteristics as well as compensating adjustments in buoyancy. Presumably the depletion of fat in spawning capelin is exceptionally rapid and compensated for by the uptake of water, hence the water/solids ratio does not have time to readjust to an optimum, resulting in the poor "condition" of the fish muscle and the low yields of meal on reduction.

From a study of their extensive data on *eviscerated* herring, as well as that of Brandes and Dietrich (1953) on herring fillets, Iles and Wood (1965) also point out that the sum of fat and water is not constant, but increases with the fat content (Fig. 1). They also found little difference between groups of herring at different maturation stages. With the present data on capelin (results for male and female fish are plotted separately in Fig. 1 because of their different pattern of changing fat content noted above), the relation of

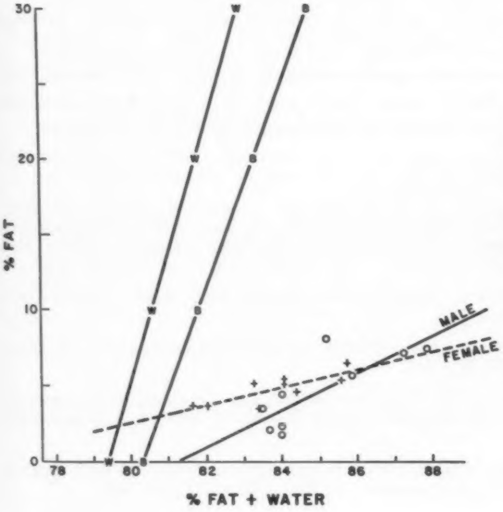

FIG. 1. Variation of fat content with fat plus water content in capelin and herring. Male (○) and female (+) whole capelin from 1966 samples Table 1. Eviscerated herring (W) from Wood's data and herring fillets (B) from Brandes and Dietrich's data as reported in table V of Iles and Wood (1965). Regression equations (Snedecor, 1956) (Y = % fat, X = % water + % fat). Male capelin Y = −98.52 + 1.212 X, 9 lots, goodness of fit, $t > .01$. Female capelin Y = −43.43 + 0.575 X, 9 lots, goodness of fit, $t > .01$.

fat to fat plus water in both male and female whole capelin differs markedly from either the eviscerated herring or the herring fillets. As fat content decreases, the decrease of fat plus water is very much greater in the capelin than in the herring samples. Although the range of fat content in the capelin samples is rather limited, the obvious difference in the pattern needs examination. Of the factors discussed by Iles and Wood (1965), one in particular should be considered in more detail. As maturation progresses, the fat and protein of the muscle tissue are converted to enlargement of the gonad tissue, and as a result the proportion of flesh weight to skeleton (and other nonmuscle constituents) decreases markedly (Idler and Bitners, 1958, on salmon; Iles and Wood, 1965, and Wilkins, 1967, on herring). Thus in the case of whole fish (as in the capelin analyses) we have the water present in the viscera and gonads (these probably have low fat content in these species — Iles and Wood, 1965) in addition to that in the flesh, skeleton, and skin, whereas in the eviscerated fish only the latter will be included. Fillets will have only muscle water. Data on proportion and composition of the various tissues would be necessary to check the results as found in Fig. 1. However, it is clear that the relation of fat to water plus fat should vary more as "condition" worsens due to maturation and spawning when whole fish are analyzed, than in eviscerated fish as used by Iles and Wood (1965). In starved fish the changes on a whole fish basis should be even greater (Wilkins (1967) examined only the fillets from starved herring).

These conclusions indicate a higher solids content for the late season capelin and thus do not support the reported lower yield of fishmeal from such late season fish. Probably other factors complicate the picture at the particular meal plant concerned. For instance, increasing difficulty in preservation of the catch due to warmer weather as the season advances and increasing delays in processing as the supply rapidly builds up could result in greater protein loss in the stickwater due to autolysis.

The ash content of Norwegian capelin meal is about 10%, about the same as herring meal (Olsen, 1967), and agrees with that expected from the 2% ash content of the one lot for which ash analysis is given in Table 1.

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Newfoundland Capelin Lipids: Fatty Acid Composition and Alterations During Frozen Storage[1,2]

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Total lipids, triglycerides, and phospholipids from several lots of beach-spawning capelin and one lot of prespawning, offshore capelin have been determined and examined in detail for fatty acid composition. Generally, but not invariably, female fish contain more fat, the difference being apparent in the triglycerides. These are also usually of higher iodine value than triglycerides in the males. During frozen storage the lipids, including triglycerides, in most samples of male capelin undergo more severe hydrolysis than in the females. It is believed that these phenomena are related to bioenergetic relationships by which the males require very rapid catabolism of depot fat and hence nonspecific hydrolysis of fatty acids, whereas the less active females show some selectivity and preferentially catabolize the longer-chain monounsaturated fatty acids.

When allowances are made for 16:1 and 18:1 being interchangeable, Newfoundland capelin triglycerides are shown to be essentially similar to eastern Atlantic commercial capelin oil, and may be distinguished by certain composition characteristics from other Canadian commercial oils of comparable iodine value. Details of fatty acid compositions are summarized for nutritional evaluation of capelin and capelin products.

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INTRODUCTION

The capelin (*Mallotus villosus*) is a circumpolar species which serves as an important food chain link between plankton and many commercially important fish such as cod (*Gadus morhua*) and Greenland turbot (*Reinhardtius hippoglossoides*). Primarily it is a bottom spawner and migrates seasonally to spawn on the bottom off the northern coasts of Norway, around Iceland, in the

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Strait of Georgia, and elsewhere. Spawn has recently been located on the Grand Banks off Newfoundland (Pitt, 1958). However, capelin are a local phenomenon along the coasts of Newfoundland and Labrador because of the more unusual mass beach spawning which occurs in these areas. The male fish school close inshore off suitable sand and gravel beaches, whereas the females school farther offshore and individually swim in when ripe to spawn with one or more males in the surf. This action has been described in varying detail (Templeman, 1948, 1966, 1968; Pitt, 1958; Winters, MS, 1966, 1968).

The capelin fishery is a major underexploited Canadian resource (Templeman, 1966). In addition to proposed reduction to meal and oil and use as mink and pet food, some interest has been shown in marketing a canned sardine-type product (Sepic and MacCallum, 1969), or dressed frozen capelin, in small amounts for domestic consumption, and also in using capelin as a raw material for the production of fish protein concentrate (FPC). Development of these uses for fish taken in the brief shore-spawning season would probably entail storage in a frozen condition for short or long periods of time. The effects of this storage on lipids and fatty acids have now been investigated, and the opportunity has been taken to compare various samples with capelin oil studied earlier in this laboratory (Ackman et al., 1963), and with a commercial Norwegian oil.

EXPERIMENTAL

Samples Examined for Proximate Composition

The proximate compositions of several different lots of beach-caught samples, including samples E, F, G, and I below, taken in the summer of 1966, are given elsewhere (MacCallum et al., 1969).

Samples for Detailed Fatty Acid Composition

(A) The benzene extract (total lipid) from 52 whole male fish collected at Torbay in 1962 as described by Ackman et al. (1963) (lipid recovery 3.35%).

(B) A benzene extract prepared at the same time from 52 additional male fish from this lot (lipid recovery 3.30%).

(C) The benzene extract from whole female fish from this lot as described by Ackman et al. (1963) (lipid recovery 3.39%).

(D) A sample of authentic Norwegian commercial capelin oil. Details of origin unknown.

(E) Code 42044 male capelin taken at Middle Cove (near St. John's) on June 24, 1966, for the Fisheries Research Board of Canada, St. John's Technological Unit, and immediately plate-frozen in packages to –30 C (–20 F), then stored at –23 C (–10 F). Six whole fish (average length 18 cm) were extracted in December 1966, with $MeOH/CHCl_3$.

(F) Code 02620 female capelin taken at the same time and place for the St. John's Technological Unit, and frozen in packages immediately. Six whole fish (average length 15 cm) were extracted in December 1966, with $MeOH/CHCl_3$.

(G) Code Y block-frozen capelin caught at Middle Cove in June 1966, and held in frozen storage (–23 C) in blocks by the St. John's Technological Unit. The outer edges of the block were sawed away leaving fish embedded in ice. $MeOH/CHCl_3$ extracts were prepared in January 1967 as follows: G-1 — the bodies (less viscera) and heads of 15 male fish, average length 15 cm; G-2 — the viscera from G-1; G-3 — the eggs from the only female fish found in lot Y.

(H) Code X (1966 summer fish) supplied by the St. John's Technological Unit but originating as commercial (–15 to –20 C) capelin frozen in blocks by Job Bros. Ltd., origin unknown.

After trimming it was possible to recover one whole female fish and parts of three others. MeOH/$CHCl_3$ extracts were prepared in February 1967, as follows: H-1 — the bodies, complete except for the roe, of one intact and parts = 1⅓ female fish; H-2 — the roe from these fish, including one whole ovary and parts of others.

(I) Male fish from sample H: I-1 — bodies (less viscera) and heads from nine fish, average length 15 cm; I-2 — the viscera from I-1.

(J) Fish caught by the *A. T. Cameron* in Trinity Bay, Nfld. (47°56′30″N, 53°31′00″W) on March 5, 1967. Frozen immediately (to −30 C), held briefly (−20 C) in the frozen state, and shipped with dry ice to Halifax for analysis. Code 49197. Extracted on March 20, 1967. J-1 — bodies (less viscera) and heads from four male fish (average length 15 cm); J-2 — viscera from fish of sample J-1.

(K) Female fish (Code 43749) from same catch as sample J. K-1 — bodies (less viscera and eggs) and heads from four fish, average length 14 cm; K-2 — viscera from K-1; K-3 — roe from K-1.

Examination of Lipids

The lipids extracted with benzene in 1962 were not examined in detail (samples A–C). The other lipids, extracted by the method of Bligh and Dyer (1959), were separated into three classes, viz. phospholipids, triglycerides, and "other" lipids by chromatography on a column (20 × 35 cm) of styrene divinylbenzene copolymer beads (200–400 mesh, Dow Chemical Co.) with benzene as the eluant (Tipton et al., 1964) monitored by a differential refractometer (Vandenheuvel and Sipos, 1961). Fraction purity was further controlled by thin-layer chromatographic examination and pooling of appropriate subfractions. Free fatty acids (FFA) were determined gravimetrically for sample E after extraction as soaps from the neutral lipid (mostly triglyceride) fraction. The results of the lipid extractions and lipid fractionations are given in Table 1.

Determination of Fatty Acid Composition

The procedures followed were essentially those employed in an analysis of herring oils (Ackman and Eaton, 1966). Where samples were adequate fatty acid compositions were determined for total lipids and for the two lipid fractions of principal interest, the phospholipids and triglycerides. Comparisons of fatty acid composition are essentially on a "type of lipid" basis in Tables 2–5. The presentation of composition data to two places of decimals is solely to show the presence of recognizable minor components. Actual error is probably $\leqslant 5\%$ for major components ($>5\%$), and $\leqslant 10\%$ for components in the 1–5% range, but may be as high as 50% for components $\ll 1\%$. All samples were hydrogenated, in most instances quantitatively, although in some instances on a microscale (Vandenheuvel, 1952), and chain lengths were compared to confirm gross accuracy. The chain lengths in the hydrogenated samples are given in Table 5 together with other summary data of interest.

RESULTS AND DISCUSSION

In addition to material on capelin cited earlier (Ackman et al., 1963), recent publication of Newfoundland material will be found in Templeman (1966, 1968), and Winters (MS, 1966) summarizes the recent biological literature. Winters (1968) also presents some fat contents from exploratory offshore fishing data. Tibbo and Humphreys (1966) discuss the occurrence of capelin in the Bay of Fundy.

In Norwegian waters there has been a very large expansion of the fishery since about 1952, annual landings now being approximately 300,000–400,000 tons. Research has shown that these fish contain 18.6–21.5% fat when in the Barents Sea, but that this level drops to about 10% as the fish move onto the Finnmark coast. Finally, there is a rapid drop from 7–10% to 1–2%

TABLE 1. Summary of sample data with lipid yields, proportions of different lipids, and iodine values calculated for fatty acids in different lipids.

Sample no.	Sex	No. of fish	Part of fish	Wt (g)	Wt % lipid	Composition of lipid in wt %			Phospholipids as % of sample	Iodine value calculated from GLC[b] data for fatty acids		
						Phospholipid	Triglyceride[a]	Other		Total lipid	Phospholipid	Triglyceride
A	M	52	Whole	...	3.35	...	...	...	...	117	...	...
B	M	52	"	...	3.30	...	...	...	...	115	...	...
C	F	56	"	...	3.39	...	...	...	...	139	...	...
D	...	...	...	...	...	...	...	...	...	109	...	...
E	M	6	Whole	284	1.90	8.8	81.9	2.9[c]	0.17	113	230	99
F	F	6	Whole	149	3.88	24.1	62.2	13.4	0.90	154	205	138
G-1	M	15	Body	472	3.04	18.8	76.9	4.1	0.57	125	250	122
G-2	M	15	Viscera	26.3	5.34	17.9	76.3	5.9	0.95	140	181	131
G-3	F	1	Roe	8.1	5.68	19.2	77.5	3.3	1.09	...	205	169
H-1	F	2.5	Body	46.6	2.88	13.4	83.4	3.2	0.39	165	216	128
H-2	F	1+	Roe	18.4	0.84[d]	...	...	...	...	176	...	...
I-1	M	9	Body	281	1.94	16.5	80.2	3.4	0.32	161	237	142
I-2	M	9	Viscera	43	4.56	23.0	70.3	6.7	1.05	168	203	155
J-1	M	4	Body	122	2.60	11.9	86.0	2.1	0.31	126	218	104
J-2	M	4	Viscera	10	10.00	15.5	78.7	5.9	1.55	116	191	101
K-1	F	4	Body	87.5	9.25	12.6	86.4	1.0	1.17	126	216	117
K-2	F	4	Viscera	5.4	4.94[d]	...	...	...	...	134	...	...
K-3	F	4	Roe	4.2	3.28[d]	...	...	...	...	162	...	...

[a] Includes minor amounts of free fatty acids in some instances.
[b] GLC = gas–liquid chomatography.
[c] Does not include 6.4% FFA analysed separately.
[d] No further analysis.

during the fishing season on the coast. Similarly, Icelandic research shows a drop from 10% fat to 3% fat during the fishing season. It is also reported that in eastern Atlantic capelin the female fish are slightly higher in fat than males caught at the same time, that the fat distribution is about 80% in the body muscle and 20% in the abdominal cavity, and that iodine values are approximately 103–116 for Norwegian oil and 98–108 for Icelandic oil (P. M. Jangaard, personal communication; Nitter-Egenaes, 1967a, b; Olsen, 1967).

FAT CONTENT AS RELATED TO SEX OF FISH

In the fat content data assembled by MacCallum et al. (1969) instances may be observed in which there is no large difference with sex in given lots of fish. When there are differences the female is normally, but not invariably, richer in fat. The data in Table 1 confirm this view (note duplication of listings) for samples A, C, E, F, G, I, J, and K (whole body figure calculated from Table 1 data). Note particularly that when there is a difference, it is usually in samples from late in the season when fat levels are low. The lipid or fat contents for the 1966 samples (MacCallum et al., 1969) range from a high of 8.1% (on June 15) to a low of 1.8% (on July 5) with an average of 4.5%, which is possibly abnormally high for Newfoundland beach-spawning capelin, although the trend conforms to eastern Atlantic observations (see above).

Winters (1968) found very little difference in fat content with sex in various trawl-caught offshore samples, although there was a possible decline in fat from the beginning of spring feeding in March (overwintering apparently does not materially affect fat) to the beginning of the spawning season. Winters also observed that older and larger capelin matured earlier and found separate schools near the beaches in late May, thus confirming earlier observations (Winters, MS, 1966) that the larger fish are the earlier to spawn, followed by smaller, more slowly maturing fish. Fat content in beach spawners could be affected by this difference, the larger fish either starting with more fat after wintering or using less in the immediate prespawning period. In this connection it is extremely important to note that in the capelin trawled in Trinity Bay on March 7, 1967, the present study found 8.8% fat in 14-cm females but only 3.2% in 15-cm males (calculated from data in Table 1). Winters (1968) reported that in capelin taken from Trinity Bay in the same survey on March 5, 1967, fat contents were respectively 12.8% for 19-cm females and 12.7% for 20-cm males. There is also a small possible exaggeration of the reduced lipid level in males, when lipid levels are already low, due to hydrolysis of phospholipids during frozen storage (see below).

In male fish, at least, the viscera are rich in fat, some 20–25% of the fat in samples I and J being in the viscera. Adequate numbers of female viscera were sampled only in the unusually fat offshore fish of K, where the viscera and roe then contained only about 5% of the total lipid. However in samples H and I the overall impression of more fat in the body of females is sustained despite the observation that in fillets cut from fish taken in 1960 (MacCallum et al., 1969) the male muscle had nearly twice the fat level of the female muscle.

When possible, serious consideration should be given to size and maturity, as noted above, in comparing fat levels. Templeman (1948) pointed out that there is little evidence that male capelin survive on the beaches to spawn more than once, whereas females may spawn several times. Winters (MS, 1966) mentions the heavy mortality among large capelin which are early on the beaches, and much of the data available for consideration is obviously based on late and therefore relatively juvenile fish, possibly including those spawning for the first time. Nitters-Engeaes (1967b) notes that in most years eastern Atlantic capelin arriving in the latter part of the Norwegian fishery are very lean and in poor condition, and in some years the failure of the fishery may be due to complete exhaustion of the fat reserves preventing the fish from reaching the normal fishing areas.

RELATIONSHIP OF SEX OF FISH TO LIPID HYDROLYSIS IN FROZEN STORAGE

In a previous examination of capelin the levels of lipid extractable from the whole bodies were about 3.3% when benzene was used as the solvent (samples A–C, Table 1). In the current study the efficient chloroform–methanol–water system of Bligh and Dyer (1959) was used for extraction purposes. Unlike some solvent systems (e.g., diethyl ether) this system totally recovers phospholipids as well as neutral lipids (triglycerides, cholesterol, cholesterol esters, hydrocarbons). It may be concluded, from a wide variety of species which have been examined, that extracts of whole fish (intact, headed, or with viscera removed), or the total muscle of fish, will yield as a minimum lipids representing approximately 0.6–1.2% of the wet weight of the sample used. When allowances are made for bone, and some other factors such as an enriched lipid content in lateral line (dark) muscle, the normal range for phospholipids in fish muscle in whole fish may be somewhat narrower, perhaps 0.6–0.8% of the wet weight of muscle (Bligh and Dyer, 1959; Dambergs, 1964; Silk and de Koning, 1964; Froines et al., 1965; Olley and Duncan, 1965; Bligh and Scott, 1966; de Koning, 1966a, b; de Koning and McMullan, 1966; Ackman, 1967; Jangaard et al., 1967; Linko, 1967; Roubal, 1967; Addison et al., 1968; Bosund and Ganrot, 1969). This range applies to fish which are fresh or which have been stored under conditions such that hydrolysis of the phospholipids had not taken place (Olley and Duncan, 1965; Bligh and Scott, 1966; Ackman, 1967; Castell et al., 1968), and is nominally independent of seasonal variations in depot fat which may be deposited in the same tissue (e.g., herring triglycerides). These phospholipids are probably intimately involved in cell membranes and function, and therefore represent residual "essential" lipid remaining when all depot lipid reserves such as triglycerides have been exhausted. Cod lipids have received particular attention (see Love, 1958; Ackman and Cormier, 1967; Olley, 1965, cited by Wilkins, 1967). Sutton (1968) examined the ion and water contents of cod muscle during starvation, and suggested that during starvation muscle phosphorus (mainly bound) decreased proportionately as water increased, but that intracellular phosphorus went through a minimum when plotted against weight fraction of cells. This is said to be part of a process by which starvation causes alterations of cell materials in two stages, catabolism of tissue components causing few important changes until water reaches 83%, at which point high molecular weight materials cannot be freely utilized and osmotically active materials are depleted, causing shrinkage of the cell. In a study of starved herring, Wilkins (1967) did not actually isolate phospholipids from the fillets studied, although he reported levels as low as 0.08% of muscle tissue, but concluded that as a proportion of total lipid the phospholipid *decreased* during the later stages of starvation. There is insufficient data to correlate the reports of Sutton and Wilkins in terms of definite lipid components, but cell *shrinkage* could permit some phospholipid to be liberated from various biomembranes.

In neutral systems of lipids it is possible to estimate free fatty acids (FFA) simply by direct titration as well as by isolation and subsequent titration or gravimetric determination. This is not true of systems rich in phospholipids, and Olley and Lovern (1960) and Lovern and Olley (1962) found that in cod flesh lipids titrated to a phenolphthalein end point two minor phospholipids (mostly phosphatidyl serine and phosphatidyl inositol) did not hydrolyse and contributed to the titration of extractable lipid to an extent equalling about 6% FFA, whereas the readily hydrolysable phosphatidyl ethanolamine contributed a diminishing absolute amount to the titration, falling from about 15% FFA in fresh lipid to about 2% at the stage when rapid lipid hydrolysis ceased. The contribution from these phospholipids to "% free fatty acid" as determined by titration in the capelin lipid studies of MacCallum et al. (1969) will therefore depend on the type of phospholipids initially present, although it is believed that these are essentially similar in composition in most marine animals, with phosphatidyl choline predominating (~60%), phosphatidyl ethanolamine and serine present in lesser amounts (~20%), and others in small proportions (see below and multiple references cited above). Many of the results listed by MacCallum et al. for FFA in lipid are slightly higher than would be the case if they were based strictly on titration of FFA alone, but in most instances correction is impractical on the basis of the data available. A differential titration with two indicators has been applied to a similar problem of FFA determination in lambs' liver (Rhodes and Lea, 1961).

Until recently it was believed that triglycerides in frozen fish muscle were less subject than phospholipids to hydrolysis by lipolytic enzymes, although in elasmobranchs, such as the dogfish *Squalus acanthias*, the FFA arose principally from the triglycerides (Olley et al., 1962; Olley and Duncan, 1965).

Takama et al. (1967) followed lipid alterations in *minced* frozen bluefin tuna (*Thunnus orientalis*). After 100 days, 61% of the triglyceride originally present had been hydrolysed (initial lipid content 1.60%, of which triglyceride was 45%). Because of the minced conditions, which facilitated oxidation, details of unsaturated fatty acid composition are difficult to follow after 10 days, but initial increases in 20:1 and 22:1 in the FFA confirm hydrolysis of triglyceride.

Indirect evidence, including accumulated mono- and diglycerides, has recently indicated that a substantial part of the FFA produced in frozen stored Baltic herring (*Clupea harengus*) came from triglycerides in addition to the normal production from phospholipid hydrolysis (Bosund and Ganrot, 1969). The FFA isolated by these workers were examined by gas-liquid chromatography and were intermediate in composition between the herring triglycerides and phospholipids in details of composition, but lacked any striking features which would associate them with the triglycerides. The FFA in Nova Scotia commercial herring oils have been shown to include certain fatty acids in proportions typical of both phospholipids and triglycerides (Addison et al., 1969). In the present detailed lipid study there was a considerable difference in lipid recovery from male and female fish of lots E and F, although both had been handled and stored in the same fashion. Moreover the amount of phospholipid was appreciably less (0.17%) in the male fish than in the female fish (0.94%), indicating that severe hydrolysis had taken place. The triglyceride component from the gel column normally includes some FFA in the latter part of the peak, and this, together with the next portion of "other" lipids to elute, was neutralized with alkali and the FFA (6.4% of the lipid) recovered by conventional means. The composition of the FFA (Table 2) displays a definite component derived from phospholipids (high 22:6ω3; see Table 4), but two other major components should be largely derived from triglyceride (high 20:1 and 22:1).

In the remaining two lots of fish in which detailed comparisons may be made (Table 1) body lipid hydrolysis has probably occurred equally in both males (I-1) and females (H-1) in one lot where the history of the sample is not well known and storage conditions may have favoured hydrolysis through temperature cycling or other handling problems. The last lot, however, provides interesting support for a differentiation of lipid hydrolysis by sex in that the time elapsed between catching and lipid examination was short (15 days) and the fish presumably well frozen. Nevertheless, the body lipid of female fish (K-1) shows apparently intact phospholipid (1.17%), whereas the body lipid from the males (J-1) shows a low level (0.31%) indicative of severe lipid hydrolysis. Although the total lipid recovery (2.60%) was low for the bodies of fish J-1, the recovery of triglyceride is evidence that the fish retained some degree of lipid reserve. Unfortunately it is not known if this lipid is truly of muscle origin or if traces of mesenteric fat were included with the extracted muscle.

The proximate composition data of MacCallum et al. (1969) indicates that in the nine 1966 samples there are four in which % FFA (by titration) is higher in the lipids from males than from females. These are four out of the five samples which have the lowest lipid contents, in both males and females, of the nine samples under consideration. In the five samples in question the

TABLE 2. Fatty acid weight per cent composition of triglycerides recovered from extracted lipids of Newfoundland capelin, compared with acids of a free fatty acid sample, and of Norwegian commercial capelin oil.

Fish portion:	Whole fish		Bodies and heads					Viscera			Roe	Free fatty acids	Norwegian capelin oil
Sex:	M	F	M	M	M	F	F	M	M	M	F	M	?
Specific sample: Fatty acid[a]	E	F	G-1	I-1	J-1	H-1	K-1	G-2	I-2	J-2	G-3	E	?
12:0	0.45	0.03	0.23	0.26	0.12	0.32	0.18	1.05	0.16	0.24	0.25	1.06	0.15
13:0	0.33	–	0.02	0.07	–	0.07	–	0.36	0.05	–	0.02	0.21	0.06
14:0	9.26	7.38	7.98	7.49	8.05	8.33	8.37	7.94	5.02	8.39	6.43	5.47	7.85
14:1ω5[b,c]	0.38	0.19	0.83	0.42	0.66	1.25	0.68	0.96	0.37	0.85	0.43	0.41	0.71
15:0	0.31	0.25	0.48	0.44	0.23	0.58	0.18	0.61	0.37	0.39	0.45	0.31	0.53
15:1[d]	0.14	–	0.19	–	0.10	0.35	–	0.36	–	0.08	0.33	0.12	0.34
16:0	8.53	11.05	9.60	11.24	8.42	8.70	9.04	8.83	11.00	8.09	14.65	7.39	11.08
16:1ω7[b]	15.10	15.00	13.92	14.02	20.13	14.00	18.62	14.52	11.59	18.17	14.65	10.34	11.14
16:2ω7	0.07	0.32	0.28	0.45	0.35	0.53	0.24	0.30	0.73	0.21	0.66	0.14	0.36
16:2ω4[e]	0.37	0.93	0.49	0.86	1.03	0.80	0.71	0.65	0.90	0.75	1.26	0.57	0.34
16:3ω4[e]	0.23	0.89	0.96	1.46	1.18	1.20	0.99	1.64	1.37	1.00	1.08	0.65	0.13
16:4ω1	–	0.35	0.89	0.95	1.66	0.80	0.94	0.87	–	1.17	0.70	–	0.38
17:0[f]	0.71	0.08	0.89	0.56	0.44	0.54	0.65	0.91	0.23	0.63	0.98	0.10	0.47
17:1ω8?	0.52	0.04	0.21	0.04	0.13	0.13	0.23	0.36	0.06	0.37	0.45	0.03	0.38
18:0	1.78	1.47	0.93	1.26	1.09	0.94	1.45	1.28	2.23	0.85	1.52	1.36	0.99
18:1ω9[b]	7.57	10.69	8.05	8.69	7.57	8.71	8.21	7.67	9.49	7.76	12.39	6.11	16.98
18:2ω6	0.41	0.76	1.15	1.57	1.10	1.92	1.13	0.78	1.42	1.09	1.92	0.33	1.69
18:3ω6	0.21	0.52	0.16	0.50	0.26	0.51	0.37	0.50	0.63	0.25	0.44	–	0.16
18:3ω3	0.06	0.17	0.29	0.65	0.28	0.76	0.31	0.09	0.47	0.51	1.30	0.17	0.43
18:4ω3	0.85	1.50	0.81	1.28	1.67	1.01	1.76	0.88	0.95	1.27	2.78	0.62	2.08

(*Continued*)

TABLE 2. Fatty acid weight per cent composition of triglycerides recovered from extracted lipids of Newfoundland capelin compared with acids of a free fatty acid sample, and of Norwegian commercial capelin oil. — (*Concluded*)

Fish portion:	Whole fish		Bodies and heads					Viscera			Roe	Free fatty acids	Norwegian capelin oil
Sex:	M	F	M	M	M	F	F	M	M	M	F	M	?
Specific sample:	E	F	G-1	I-1	J-1	H-1	K-1	G-2	I-2	J-2	G-3	E	?
Fatty acid[a]													
19:0 and 19:1	0.08	0.32	0.03	0.27	0.04	0.07	0.20	0.13	0.31	0.41	0.55	0.05	0.28
20:1ω9[b]	22.72	14.82	18.15	14.57	20.45	15.66	18.31	15.77	12.84	21.62	6.25	12.57	18.89
20:2ω6	0.13	0.09	0.45	0.26	0.22	0.23	0.54	0.82	0.59	0.51	0.18	0.20	0.17
20:3ω6	0.06	0.09	0.06	0.04	0.02	0.06	0.11	0.09	–	–	0.14	0.07	0.08
20:4ω6[g]	0.07	0.06	0.26	0.24	0.11	0.37	0.17	0.76	1.05	0.24	0.36	0.71	0.10
20:4ω3	–	0.38	0.28	0.21	0.24	0.38	0.26	0.71	0.43	0.28	0.81	0.32	0.25
20:5ω3	6.11	10.09	7.11	9.63	4.94	6.66	7.39	8.46	13.50	4.23	11.08	15.23	4.61
21:5ω2?	–	0.19	0.28	0.37	0.30	0.22	0.47	0.68	0.40	0.45	0.22	0.37	0.23
22:1ω11[b]	19.60	12.67	17.74	13.38	15.62	16.83	14.64	12.76	11.67	18.25	4.11	20.55	14.71
22:2ω6?	–	–	0.15	0.11	0.22	0.58	–	0.82	0.12	0.29	–	–	0.06
22:4ω6	0.03	–	0.02	0.10	–	–	–	–	0.06	–	0.08	–	–
22:5ω6	–	0.19	0.03	0.03	–	0.03	–	0.30	–	–	–	0.16	0.06
22:5ω3	0.39	1.01	0.65	0.81	0.64	0.82	0.63	0.76	1.46	0.35	1.08	1.58	0.30
22:6ω3	2.64	7.28	5.65	7.28	2.25	6.18	2.60	5.42	9.76	1.08	11.67	12.32	2.99
24:1ω?	0.89	1.19	0.75	0.47	0.48	0.46	0.72	0.99	0.66	0.22	0.81	0.47	1.02

[a] Chain length, number of double bonds, position relative to terminal methyl group.
[b] Probable principal isomer, other isomers likely to be present.
[c] Includes 4,8,12-trimethyltridecanoic acid.
[d] Includes Iso 16:0 and 2,6,10,14-tetramethylpentadecanoic acid.
[e] Includes Iso and Anteiso 17:0.
[f] Includes 3,7,11,15-tetramethylhexadecanoic acid.
[g] Includes 20:3ω3.

lipid in the male fish was lower than in the females, whereas in the remaining four the reverse was true.

The phospholipid composition data (Table 3) should be reviewed against the background of muscle lipid composition data for various species (see Olley and Duncan (1965) and other references cited above for phospholipids), keeping in mind that in most species phosphatidyl choline will be about 60% of the muscle phospholipid and phosphatidyl ethanolamine about 20%. There is a strong resemblance to the phospholipid fatty acid composition reported for most true marine species such as Nova Scotia cod (Addison et al., 1968) and herring (Drozdowski and Ackman, 1969), but certain aspects differ from those detailed for the Baltic herring which lives in a somewhat different environment (Linko, 1967). The palmitic/stearic ratio (ranging from 7 to 10) in the capelin muscle phospholipids is indicative of a high level of phosphatidyl choline. The phospholipids from viscera and roe differ somewhat from the muscle phospholipid in fatty acid composition. This is not entirely unusual as shown by an analysis of cod liver phospholipids (Ackman et al., 1967), which differ in fatty acid detail from cod flesh phospholipids. It appears likely that hydrolysis of phospholipids in frozen capelin is nonselective since there is little difference in comparative fatty acid compositions of lipids recovered even after severe hydrolysis (e.g., E and F, J-1 and K-1).

It is therefore possible to view typical higher fat (in females) with typical higher % FFA formation (in males) as two aspects of one development: the catastrophic mobilization of depot fat as a source of energy in the later stages of sexual maturation and during spawning. The parallel with the fat utilization during riverine migration of the Pacific sockeye salmon *Oncorhynchus nerka* (Idler and Bitners, 1960) is apparent and the inclusion *at one time* of capelin in the Salmonidae instead of the Osmeridae (Winters, 1966) is therefore of particular interest. Further interesting aspects of this are the behavioural patterns of prolonged male activity in the surf while awaiting the females (Templeman, 1948), and the morphological change of the development of the distinctive "spawning ridge" exhibited by the male for a few weeks up to and including the short spawning season which is unusual in marine species. As a hypothesis it is suggested that rapid development of a high % FFA in frozen capelin, and more especially male capelin, is associated with enzyme systems intended for rapid depot fat hydrolysis and catabolism peculiar to capelin in the spawning period. However, there are possibly other factors involved, such as the tendency of the males to feed up to a later stage in gonad development (Leim and Scott, 1966) and the distinctive preference of the capelin for very cold water except during the actual spawning period.

Olley and Watson (1962) have observed that in iced (gutted) herring a clear distinction must be made between FFA produced in the muscle proper, and that produced in the subcutaneous fat layer. In the muscle, FFA arose solely from phospholipid under these particular experimental conditions, but in the skin and subcutaneous fat approximately half of the FFA arose

TABLE 3. Fatty acid weight per cent composition of phospholipids recovered from extracted lipids of Newfoundland capelin.

	Whole fish		Bodies and heads					Viscera			Roe
Sex:	M	F	M	M	M	F	F	M	M	M	F
Specific sample: Fatty acid[a]	E	F	G-1	I-1	J-1	H-1	K-1	G-2	I-2	J-2	G-3
12:0	–	0.05	–	0.30	0.21	0.66	–	–	0.28	0.16	0.18
13:0	–	–	–	–	–	0.34	–	–	–	–	0.08
14:0	2.16	4.45	1.77	2.23	2.05	1.92	1.86	2.90	2.81	2.35	3.26
14:1ω5[b,c]	–	–	0.33	0.26	0.21	0.44	0.21	0.46	0.42	0.23	1.00
15:0	0.18	0.80	0.59	0.27	0.30	0.44	0.56	0.43	0.75	0.37	0.92
15:1[d]	–	–	0.15	–	–	0.06	–	0.12	–	–	0.42
16:0	21.16	22.85	17.55	17.50	20.91	15.06	20.45	23.43	19.76	20.25	21.22
16:1ω7[b]	4.89	7.30	4.52	5.31	6.50	5.46	6.73	6.36	5.68	7.50	7.04
16:2ω7	–	0.25	0.82	0.67	0.45	1.08	0.98	1.28	1.34	0.41	1.18
16:2ω4[e]	0.32	0.86	0.20	0.13	0.82	0.77	0.49	0.85	0.53	0.22	0.26
16:3ω4[e]	0.16	0.45	1.15	2.38	0.54	2.18	3.04	0.46	2.53	0.69	1.30
16:4ω1	–	0.40	0.21	–	0.50	–	0.21	0.40	0.56	0.09	0.83
17:0[f]	0.05	0.16	0.93	0.84	0.27	0.65	0.96	0.54	1.17	1.07	1.70
17:1ω8?	–	0.06	0.15	0.07	0.13	0.22	0.10	0.43	0.06	0.28	0.36
18:0	2.62	2.35	2.06	2.57	2.55	3.26	3.02	3.05	3.49	3.04	1.87
18:1ω9[b]	10.47	11.73	9.04	10.29	11.37	13.37	11.79	12.14	11.69	11.67	9.79
18:2ω6	0.43	0.48	1.02	1.11	1.02	1.31	1.55	0.70	1.34	1.10	0.77
18:3ω6	–	–	0.40	1.59	0.35	1.15	0.24	0.82	2.21	0.66	0.92

18:3ω3	0.22	0.04	0.52	0.21	0.65	0.55	0.73	0.35	0.72	0.69	0.97
18:4ω3	0.33	0.06	0.39	0.19	0.59	0.22	0.63	0.13	0.56	0.62	0.56
19:0 and 19:1	0.06	0.05	0.05	0.16	0.17	0.37	0.15	0.12	0.23	0.08	0.31
20:0	–	–	–	–	–	–	–	0.08	0.06	0.16	–
20:1ω9[b]	4.30	3.34	2.82	3.27	3.06	3.32	2.50	4.61	3.99	5.78	2.15
20:2ω6	0.05	0.10	–	0.12	0.13	0.08	0.34	0.19	0.23	0.20	0.12
20:3ω6	0.06	0.03	0.02	–	–	0.06	–	0.09	–	–	0.05
20:4ω6[g]	0.52	0.53	0.52	0.48	0.54	0.62	0.47	0.90	0.97	1.22	0.43
20:4ω3	0.10	0.14	0.24	0.32	0.30	0.31	0.31	0.15	0.25	0.28	0.23
20:5ω3	15.92	16.02	14.91	12.35	13.46	10.05	10.47	12.02	11.90	14.74	16.79
21:5ω2?	0.28	0.24	0.52	0.49	0.38	0.20	0.24	0.29	0.32	0.40	0.23
22:1ω11[b]	3.30	1.69	2.22	3.16	2.70	3.46	1.51	3.99	1.59	5.26	1.75
22:2ω6?	–	–	0.02	–	–	2.01	–	0.15	–	0.13	–
22:4ω6	–	–	–	–	–	0.12	–	–	0.17	–	0.25
22:5ω6	–	–	0.13	–	0.10	–	–	0.10	–	–	–
22:5ω3	1.90	2.40	2.48	1.89	2.10	1.91	1.55	1.69	2.12	1.92	2.08
22:6ω3	29.87	23.17	33.59	31.62	26.51	27.05	27.91	19.35	21.52	17.72	19.65
24:1ω?	0.65	–	0.58	0.22	1.13	1.30	1.00	1.42	0.75	0.71	1.33

[a] Chain length, number of double bonds, position relative to terminal methyl group.
[b] Probable principal isomer, other isomers likely to be present.
[c] Includes 4,8,12-trimethyltridecanoic acid.
[d] Includes Iso 16:0 and 2,6,10,14-tetramethylpentadecanoic acid.
[e] Includes Iso and Anteiso 17:0.
[f] Includes 3,7,11,15-tetramethylhexadecanoic acid.
[g] Includes 20:3ω3.

from triglycerides and the balance from phospholipids. Histochemical examination of frozen stored capelin (males from same lot as sample A) did not indicate a subcutaneous fat layer comparable with that found in frozen stored herring run as controls. The capelin had about 3.5% total fat, the herring about 8.1%. We therefore do not believe that subcutaneous fat plays a significant role in FFA formation in capelin. Unfrozen whole capelin would of course also show FFA development through other lipolytic processes. Recent Norwegian experiments on large-scale holdings (tanks of capacities of up to 150 tons) of unfrozen capelin with and without preservatives also demonstrate the effect of temperature (Lohne, 1968). At a starting temperature of +3 C, the FFA reached nearly 6% in 7 days, but icing to reduce the initial temperature to −0.6 C cut this FFA development effectively in half for the same storage period, and generally nearly doubled keeping time.

TRIGLYCERIDE FATTY ACID COMPOSITIONS AS RELATED TO SEX OF FISH

A commercial oil should be essentially triglyceride (>95%) with 1-2% nonsaponifiable material, and if of good quality has <3% FFA. There may be minor amounts of mono- and diglycerides, but little or no phospholipid. In the present study the lipid contents of the fish were in fact so low that no appreciable amount of commercial oil could have been produced. However, the isolation of a triglyceride fraction containing in some instances up to 5% FFA (estimated visually from thin-layer chromatography) does permit some consideration of real and hypothetical oil properties.

The iodine value provides a quick guide to the nature of the fatty acids in a marine oil, since in general the level of saturated fatty acids is relatively constant and the interchange of monoethylenic acids, especially 20:1 and 22:1, with the highly unsaturated polyethylenic fatty acids, is rapidly reflected in iodine values (Ackman, 1966; Ackman and Eaton, 1966).

In a previous examination of lipids from whole capelin (Ackman et al., 1963) it was found that the extract or "oil" from the females had a higher iodine value (144) than that from males (125). This was ascribed to the presence of highly unsaturated lipid from the roe, although it was thought that this would include a substantial amount of phospholipid or fatty acid from phospholipid. Referring to the data in Table 1 it is evident that the whole fish data for samples E and F are in agreement with the previous results, but more remarkably the triglyceride portion of the lipid from females is primarily responsible for the difference. In comparable samples J-1 and K-1 the body triglycerides show the same trend, although this is not observed in the body lipids from H-1 and I-1. It is conceivable that the extensive hydrolysis of phospholipids in I-1 could have contaminated the triglyceride fraction with FFA from this source (note the typical phospholipid high 16:0 and $22:6\omega3$ of I-1 analysis in this group of lipids from bodies and heads in Table 2).

In the males the visceral lipid (which may be a substantial part of the total lipid in the fish; see above) reflects the iodine values of the corresponding body triglycerides. In the three samples where this examination was made, respective calculated iodine values were 131 and 122, 155 and 142, and 101 and 104. This observation suggests that both of these triglycerides are depot fat lipids and correspond to a common pool of fatty acids. In the females it is not unreasonable to expect that the roe triglyceride would similarly correspond to the body triglyceride in respect to fatty acid composition, particularly if the triglyceride for the roe is mostly mobilized in the latter stages of maturation. Unfortunately in the present study the scarcity of female material owing to the ratios of sexes spawning on the beach made a study of this point impossible. However, it follows from the hypotheses based on % depot fat and % FFA that the female capelin should catabolize preferentially certain fatty acids of low iodine value associated with energy reserves such as the 20:1 and 22:1 fatty acids (Table 4, whole fish results). In nonfeeding trout this results in accumulation of $20{:}5\omega3$ and $22{:}6\omega3$ (Kaneko et al., 1967). The males would be less specific due to their higher level of activity and requirement of a more rapid lipid hydrolysis and fatty acid catabolism (Havel et al., 1967; Kreuger et al., 1968; Schonfield and Rodriquez, 1968). This would result in a higher iodine value fat for the females at a given time, and it must be supposed that the roe triglycerides reflect this fatty acid composition of depot fat in the female. This would explain why the female capelin have more highly unsaturated lipids, including the roe triglycerides as well as the roe phospholipids where high iodine value fatty acids would normally be expected. If this view is not correct, then it is necessary to suppose that highly unsaturated fatty acids are specifically organized into triglyceride in the roe to confer some unknown metabolic benefit on the developing young.

Irrespective of the above views, the present study confirms that capelin oils will likely have different iodine values depending on the ratios by sex in the fish processed, with the females providing generally higher values.

OTHER ASPECTS OF FATTY ACID COMPOSITIONS

The iodine value (109) of the Norwegian commercial capelin oil (sample D) is comparable with the triglyceride values of samples J-1 and K-1 (respectively 104 and 117). This suggests that oil produced from Newfoundland offshore pre-spawning capelin should be comparable with that from eastern Atlantic capelin. Very similar values are found (Table 2) for individual major fatty acids 14:0, 20:1, $20{:}5\omega3$, 22:1, and $22{:}6\omega3$ (Table 2), and also for the C_{14}, C_{20}, and C_{22} chain lengths (Table 5). However, the acids 16:1 and 18:1 and the corresponding chain lengths differ substantially. Referring to Table 2 in detail, there is a small difference in 16:0 levels, but it is evident that the totals $\Sigma 16{:}1+18{:}1$ (respectively 27.7, 26.8, and 28.1% in J-1, K-1, and the Norwegian oil) are very close. It has been suggested previously that these two acids are interchangeable in oils from herring and perhaps other clupeids (Ackman and Eaton, 1966), and this is evidently also the case in capelin oils of different origins. The differences in the C_{16} and C_{18} chain length totals (Table 5) are thus explained. As always it is hazardous to base generalizations on one sample but in this instance there are no other unexpected features in the eastern and western Atlantic oils.

TABLE 4. Fatty acid weight per cent composition of total lipids extracted from Newfoundland capelin with benzene (samples A–C) or MeOH/CHCl$_3$ (samples E–K).

Fish portion:	Whole fish					Bodies and heads					Viscera				Roe	
Sex:	M	M	M	F	F	M	M	M	F	F	M	M	M	F	F	F
Specific sample:	A	B	E	C	F	G-1	I-1	J-1	H-1	K-1	G-2	I-2	J-2	K-2	H-2	K-3
Fatty acid^{a}																
12:0	0.17	0.22	0.23	0.15	0.09	0.15	0.62	0.14	1.11	0.22	0.13	0.99	0.12	0.12	2.35	0.31
13:0	0.05	0.07	0.07	0.05	0.01	0.07	0.18	0.02	0.13	–	0.04	0.21	–	0.03	0.14	0.10
14:0	7.44	7.53	7.27	6.65	6.33	7.66	6.02	5.64	6.81	10.04	7.50	5.51	7.25	6.22	5.74	9.05
14:1ω5^{b,c}	0.46	0.63	0.57	0.42	0.49	0.58	1.36	0.68	0.77	1.10	0.73	1.11	0.61	0.53	0.60	0.92
15:0	0.50	0.38	0.20	0.44	0.43	0.42	0.46	0.24	0.26	0.68	0.45	0.56	0.28	0.25	0.49	0.61
15:1^{d}	0.40	0.21	0.09	0.23	0.17	0.17	0.17	0.09	0.16	–	0.31	0.14	0.10	0.13	0.29	0.49
16:0	9.69	9.32	9.42	10.13	12.36	9.76	14.88	9.18	12.53	9.80	10.67	11.62	9.29	10.30	13.77	13.36
16:1ω7^{b}	13.06	13.84	12.04	13.09	12.17	11.95	14.03	16.46	10.38	18.08	12.05	9.08	14.70	13.27	15.39	19.42
16:2ω7	0.16	0.26	0.14	0.14	0.41	0.18	0.42	0.21	0.60	0.36	0.59	0.43	0.16	0.56	0.72	0.36
16:2ω4^{e}	0.46	0.40	0.48	0.41	1.24	0.93	0.74	0.79	0.42	1.16	0.73	1.12	0.17	1.17	1.01	1.31
16:3ω4^{e}	1.34	0.55	0.55	0.69	0.55	0.77	1.25	0.92	0.61	2.07	1.10	1.43	0.37	2.18	2.31	3.23
16:4ω1	0.66	0.54	0.56	0.42	0.34	0.66	0.47	1.30	–	1.59	1.13	–	0.80	0.96	0.72	1.71
17:0^{f}	1.36	0.89	0.24	0.87	0.18	0.21	1.02	0.42	0.64	0.96	0.78	0.14	0.87	0.19	0.51	0.69
17:1ω8?	0.45	0.21	0.08	0.21	0.22	0.12	0.11	0.09	0.05	0.27	0.25	0.07	0.32	0.05	0.12	0.62
18:0	1.52	1.60	2.00	1.44	1.51	1.34	1.69	1.56	1.85	1.82	1.45	2.43	1.10	1.27	1.27	1.29
18:1ω9^{b}	7.79	8.04	8.33	9.19	10.47	7.93	8.97	9.00	9.53	8.81	7.76	9.54	8.20	8.78	10.96	9.09
18:2ω6	0.83	0.67	0.59	0.75	0.58	1.10	1.57	1.01	2.55	1.56	0.86	1.70	0.99	1.16	1.54	1.60

18:3ω6	0.13	0.30	–	0.12	0.57	0.26	0.54	0.20	0.41	0.82	0.55	0.94	0.72	1.01	1.49	2.09
18:3ω3	0.35	0.40	0.09	0.51	0.34	0.18	0.28	0.23	0.32	1.03	0.31	0.57	0.52	0.52	0.91	1.21
18:4ω3	0.96	1.01	0.70	1.49	2.52	0.71	0.77	1.11	0.88	2.26	0.84	0.97	1.11	1.11	1.97	3.07
19:0 and 19:1	0.28	0.33	0.11	0.24	0.09	0.09	0.23	0.10	0.30	0.24	0.08	0.73	0.19	0.17	0.48	0.21
20:0	–	–	–	–	–	–	0.06	–	–	–	–	–	–	–	–	–
20:1ω9[b]	21.45	21.13	20.86	16.84	12.27	16.51	9.09	20.70	10.97	14.56	16.95	11.01	19.81	17.73	5.43	4.60
20:2ω6	0.20	0.05	0.83	0.07	0.13	0.26	0.34	0.39	0.07	0.33	0.26	0.47	0.90	0.29	0.15	0.11
20:3ω6	0.07	0.01	–	0.03	0.05	–	–	–	–	–	0.06	0.11	–	0.55	0.16	0.04
20:4ω6[g]	0.17	0.17	0.17	0.20	0.31	0.17	0.60	0.21	1.45	0.20	0.44	0.84	0.75	0.33	0.42	0.24
20:4ω3	0.22	0.12	0.10	0.20	0.25	0.11	0.23	0.20	0.67	0.65	0.55	0.41	1.01	0.08	0.48	1.97
20:5ω3	6.03	6.45	6.26	10.10	10.30	7.58	9.77	6.67	10.11	7.92	9.91	12.51	7.43	7.22	12.98	10.05
21:5ω2?	0.23	0.10	0.52	0.41	0.37	0.43	0.14	0.41	0.21	0.31	0.35	0.43	0.40	0.38	0.36	0.72
22:1ω11[b]	16.89	17.60	20.39	14.54	11.78	16.37	7.72	15.38	10.54	9.11	12.14	10.44	17.91	14.44	2.83	1.87
22:2ω6?	0.22	0.08	–	0.02	–	0.15	0.50	0.56	–	0.24	0.20	0.75	0.60	0.68	0.87	0.36
22:4ω6	0.02	–	0.27	0.04	–	–	–	–	0.06	–	–	–	–	–	–	–
22:5ω6	0.02	0.02	–	0.11	0.09	0.04	0.11	–	–	–	0.13	0.12	–	–	0.13	–
22:5ω3	0.87	0.71	0.63	1.00	1.19	0.58	1.12	0.34	1.08	0.55	0.99	1.07	0.52	0.82	1.50	0.77
22:6ω3	4.83	5.27	5.32	7.88	10.99	11.68	13.69	4.63	14.32	2.84	8.59	11.87	2.34	6.18	11.51	7.57
24:1ω?	0.72	0.90	0.90	0.92	1.20	0.90	0.85	1.12	0.37	0.44	1.10	0.66	0.45	1.21	0.33	0.96

[a] Chain length, number of double bonds, position relative to terminal methyl group.
[b] Probable principal isomer, other isomers likely to be present.
[c] Includes 4,8,12-trimethyltridecanoic acid.
[d] Includes Iso 16:0 and 2,6,10,14-tetramethylpentadecanoic acid.
[e] Includes Iso and Anteiso 17:0.
[f] Includes 3,7,11,15-tetramethylhexadecanoic acid.
[g] Includes 20:3ω3.

Table 5. Chain length compositions of fatty acids in lipid samples as determined from hydrogenated methyl esters, and summary composition data derived from detailed analyses (Tables 2–4).

Specific sample:	Total lipid																	Commercial Norwegian oil
Principal fatty acids in hydrogenated sample[a]	A	B	C	E	F	G-1	G-2	G-3	H-1	H-2	I-1	I-2	J-1	J-2	K-1	K-2	K-3	
C_{12}	0.3	...	0.1	0.3	0.2	0.2	0.2	...	–	2.4	0.6	0.8	–	1.0	0.6	–	–	...
C_{13}	TRA[b]	...	TRA	TRA	TRA	0.1	TRA	...	–	TRA	0.2	TRA	–	–	–	–	–	...
C_{14}	7.2	...	6.6	7.8	7.8	7.4	8.1	...	8.4	6.5	7.4	5.7	7.0	8.4	11.6	13.3	9.9	...
C_{15}	0.5	...	0.4	0.6	0.7	0.4	0.7	...	0.2	1.2	0.6	0.7	0.3	0.7	1.0	1.5	1.1	...
C_{16}	23.3	...	22.6	23.9	29.7	23.1	25.0	...	24.6	33.7	31.8	24.2	28.4	26.4	34.0	38.6	27.4	...
C_{17}	0.8	...	0.3	0.7	0.6	0.6	0.8	...	0.5	0.8	1.1	0.7	0.4	0.4	0.6	1.2	0.5	...
C_{18}	10.3	...	12.8	10.3	14.3	10.2	10.4	...	13.7	17.5	13.8	15.5	13.7	10.9	15.5	14.5	12.6	...
C_{19}	0.1	...	0.1	0.1	0.2	0.1	0.2	...	0.5	0.3	0.2	0.2	0.1	0.1	0.2	0.6	0.1	...
C_{20}	30.0	...	29.7	27.7	23.0	26.1	28.7	...	22.6	20.0	20.1	26.1	28.1	27.0	23.5	18.6	25.7	...
C_{21}	0.3	...	0.3	0.2	0.2	0.2	0.5	...	0.1	0.4	0.1	0.6	0.4	0.1	0.3	0.3	0.3	...
C_{22}	24.9	...	26.0	27.0	21.9	28.3	24.5	...	26.5	17.0	23.1	24.9	20.2	24.2	12.2	10.8	21.6	...
C_{24}	1.1	...	1.1	1.4	1.1	0.8	1.3	...	0.2	0.5	0.8	1.1	1.5	1.2	0.6	0.6	0.7	...
Exptl																		
Iodine value hydrogenation[c]	126	125	147	121	155	128	131	...	152	163	134	144	117	115	124	126	–	...
Σ saturated acids	20.7	20.1	19.7	19.4	20.9	19.6	21.0	...	23.1	24.3	24.9	21.5	17.2	18.9	23.5	18.4	25.4	...
Σ monounsaturated acids	61.5	62.9	55.7	63.4	48.9	54.6	51.4	...	43.1	36.4	42.5	42.8	63.6	62.3	52.6	56.4	38.2	...
Σ polyunsaturated acids	17.8	17.1	24.6	17.2	30.2	25.8	27.6	...	33.8	39.3	32.5	35.7	19.2	18.8	23.9	25.2	36.4	...
Calculated																		
Σ polyunsaturated acids[d]	18.7	18.0	25.7	17.3	30.3	21.7	26.1	...	33.9	37.3	32.7	34.7	21.3	18.3	21.4	24.3	33.3	...

Triglycerides																	
C_{12}	...	...	...	0.2	0.3	0.8	1.0	–	0.3	...	–	–	–	–	0.4	...	...	0.1
C_{13}	...	...	...	–	TRA	–	–	–	–	...	–	–	–	–	–	...	...	...
C_{14}	...	...	...	10.0	8.1	8.7	9.1	6.6	11.2	...	8.1	5.8	7.0	10.1	9.0	...	...	9.1
C_{15}	...	...	...	0.7	0.7	0.5	0.9	0.7	0.7	...	1.0	1.0	0.3	0.8	1.0	...	...	0.6
C_{16}	...	...	...	26.0	27.3	26.0	26.4	33.6	24.7	...	27.8	25.4	32.4	31.8	29.2	...	...	22.9
C_{17}	...	...	...	0.9	0.7	0.8	1.0	0.9	0.7	...	0.9	0.8	0.4	0.3	0.5	...	...	0.6
C_{18}	...	...	...	9.4	15.8	11.1	11.5	20.7	12.1	...	13.0	15.2	12.7	11.1	13.5	...	...	22.0
C_{19}	...	...	...	TRA	0.1	TRA	0.3	0.3	0.2	...	TRA	0.1	TRA	0.1	0.4	...	...	0.4
C_{20}	...	...	...	30.4	24.7	27.2	27.6	17.6	24.7	...	24.4	27.7	26.1	26.8	26.2	...	...	25.1
C_{21}	...	...	...	–	0.3	0.1	0.5	–	0.4	...	0.4	0.4	0.4	0.2	0.4	...	...	0.4
C_{22}	...	...	...	22.2	21.5	24.8	20.7	18.7	24.5	...	21.7	22.7	19.2	17.9	18.5	...	...	17.6
C_{24}	...	...	...	0.6	1.0	0.7	0.9	0.4	0.9	...	1.3	0.7	1.5	0.7	1.0	...	...	1.2
Exptl																		
Iodine value hydrogenation[c]	...	...	...	101	139	109	139	175	109	...	128	143	111	95	105	...	...	113
Σ saturated acids	...	...	...	21.4	20.3	20.1	21.0	24.3	19.5	...	21.3	19.2	18.4	18.6	19.8	...	...	21.1
Σ monounsaturated acids	...	...	...	67.0	54.9	59.9	53.5	40.0	57.5	...	51.9	47.0	65.2	67.7	61.6	...	...	64.4
Σ polyunsaturated acids	...	...	...	11.6	24.8	20.0	25.5	35.7	23.0	...	26.8	33.8	16.4	13.7	18.6	...	...	14.4
Calculated																		
Σ polyunsaturated acids[d]	...	...	...	12.8	25.4	20.4	25.8	35.4	22.3	...	26.5	31.7	14.7	13.5	18.7	...	...	15.0
Phospholipids																		
C_1	...	...	...	...	–	–	–	–	0.5	...	–	–	–	–	–	...	...	...
C_{13}	...	...	...	...	–	–	–	–	–	...	–	–	–	–	–	...	...	...
C_{14}	...	...	...	...	4.3	2.9	2.8	4.2	2.3	...	3.0	2.9	2.7	5.5	2.4	...	...	...
C_{15}	...	...	...	...	0.5	0.7	0.7	0.8	0.7	...	0.6	0.6	0.8	1.1	0.4	...	...	...
C_{16}	...	...	...	...	30.9	24.4	31.3	31.7	22.4	...	25.3	29.4	28.2	31.9	32.3	...	...	...
C_{17}	...	...	...	...	0.3	0.4	1.2	1.6	1.0	...	0.6	0.9	0.7	0.8	1.1	...	...	...

(Continued)

TABLE 5. Chain length compositions of fatty acids in lipid samples as determined from hydrogenated methyl esters, and summary composition data derived from detailed analyses (Tables 2–4). — (*Concluded*)

Specific sample: Principal fatty acids in hydrogenated sample[a]	Total lipid A	B	C	E	F	G-1	G-2	G-3	H-1	H-2	I-1	I-2	J-1	J-2	K-1	K-2	K-3	Commercial Norwegian oil
C_{18}	...	...	...	...	16.0	13.1	17.4	14.8	19.5	...	15.0	19.4	15.8	15.3	18.9	...	...	...
C_{19}	...	...	...	...	0.1	0.1	TRA	0.3	0.4	...	0.3	0.2	0.3	0.2	0.4	...	...	...
C_{20}	...	...	...	...	21.4	19.6	19.2	21.2	15.0	...	17.7	18.8	17.6	22.5	14.7	...	...	...
C_{21}	...	...	...	...	0.3	0.6	0.3	0.5	0.2	...	0.7	0.2	0.7	0.1	0.3	...	...	...
C_{22}	...	...	...	...	27.8	37.7	26.0	23.1	36.8	...	36.3	26.4	32.0	21.6	28.4	...	...	...
C_{24}	...	...	...	...	–	0.4	1.0	1.7	1.2	...	1.2	1.2	1.1	1.0	1.1	...	...	...
Exptl																		
Iodine value hydrogenation[c]	...	...	...	218	191	218	160	210	210	...	254	180	197	179	204	...	...	...
Σ saturated acids	...	...	...	26.2	30.7	22.9	30.4	29.2	22.3	...	23.7	28.3	26.3	27.4	26.8	...	...	...
Σ monounsaturated acids	...	...	...	23.7	24.2	19.9	29.6	24.2	28.0	...	22.7	24.4	25.3	31.5	24.0	...	...	...
Σ polyunsaturated acids	...	...	...	50.1	45.1	57.2	40.0	46.6	49.7	...	53.6	47.3	48.4	41.1	49.2	...	...	...
Calculated																		
Σ polyunsaturated acids[d]	...	...	...	50.7	46.5	60.8	38.9	46.6	50.1	...	56.7	45.9	50.7	42.2	50.1	...	...	...

[a] Minor components lumped into adjacent larger peaks.
[b] TRA = trace.
[c] Microscale in many instances (Vandenheuvel, 1952).
[d] From ester iodine value (Ackman and Eaton, 1966).

Should a capelin reduction industry be established, it may at times be necessary to distinguish Newfoundland capelin oil from other marine oils of comparable iodine value produced in the western Atlantic area. Primarily the two samples of triglycerides J-1 and K-1 represent reasonable prospects as commercial oils. Comparison of recent data on herring oils (Ackman and Eaton, 1966; Ackman et al., 1967), Greenland (Newfoundland) turbot oil (*Reinhardtius hippoglossoides*) (Ackman et al., 1967), and redfish oils (*Sebastes* sp.) (Ackman and Ke, 1968) with the capelin oils show that there is little doubt about herring oil because of its unique chain length composition ($C_{22} \gg C_{20} > C_{16} \gg C_{18}$), the ratio $C_{22} \gg C_{20}$ being typical of clupeid oils (Lambertsen and Braekkan, 1965). The redfish oils have their typical reddish-orange colour which may occasionally be found in oils from other species feeding on crustacean zooplankters, and an undistinguished chain length composition ($C_{16} \geqslant C_{20} \geqslant C_{22} \geqslant C_{18}$). The turbot oil ($C_{16} \gg C_{20} > C_{18} \geqslant C_{22}$) and capelin oil ($C_{16} \gg C_{20} > C_{22} > C_{18}$) are similar (the turbot at times feeds heavily on capelin) except for the reversed C_{22} and C_{18} figures, but in detail 16:1 and 18:1 are of the same magnitude in the turbot oil whereas 16:1 > 18:1 is apparently normal in Newfoundland capelin if not in the eastern Atlantic oil. However, the numbers of samples available for comparison are limited except in the herring oils.

The overall total fatty acid compositions (Table 4) are intended as a guide to nutritional aspects when utilizing whole or dressed capelin, or parts thereof. Generally speaking, there is nothing unusual apparent in this data, and from a fatty acid point of view the nutritional characteristics should be similar to those of comparable products prepared from herring (see also data for types of acids given in Table 5; and fillet proximate composition data of MacCallum et al. (1969)).

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Reproductive Behaviour of the Blackspotted Stickleback, *Gasterosteus wheatlandi*

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The reproductive biology of the blackspotted stickleback (*Gasterosteus wheatlandi*) closely parallels that reported elsewhere for *G. aculeatus*, with certain noteworthy exceptions. *Gasterosteus wheatlandi* nests in brackish water of low salinity rather than in fresh water. The mature males are gold on the dorsal and lateral surfaces and white on the ventral surface with an irregular series of black spots along the ventro-lateral surfaces of the tail stalk, in contrast to the blue and red highlights of mature colouration in *G. aculeatus* males. There are marked differences also in the behaviour by which males of the two species lead females to the nest. In the blackspotted stickleback leading consists in an intricate series of movements along the substrate, the male in a head-down position and quivering, the female in a head-up posture with her snout touching the male between his pelvic spines; in contrast, *G. aculeatus* males simply swim to the nest entrance, the female following with no physical contact. Reproductive isolation between the two species probably depends on breeding habitat separation and female recognition of species-specific male colour patterns. When these fail, incompatible leading behaviours constitute an effective block to interspecific hybridization. Parental fanning in *G. wheatlandi* resembles that in *G. aculeatus* except that average bout duration is substantially less. Vertical quivering, a post-spawning activity of unknown function, is unique to *G. wheatlandi*.

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INTRODUCTION

IN EACH SPECIES of the stickleback family Gasterosteidae, the male constructs a nest, defends the nesting territory from intruders, courts and spawns one or more females, then cares for the developing eggs and young. The purpose of this study is to provide a description, chiefly qualitative, of reproductive behaviour in the blackspotted stickleback *Gasterosteus wheatlandi* (Putnam).

This species was studied for several reasons. First it is closely related to the more familiar threespine, *G. aculeatus*. Morphologically the two are easily confused. *Gasterosteus wheatlandi* differs from *G. aculeatus* in lacking lateral bony plates on the posterior part of the body and keels on the caudal peduncle, and in having prominent cusps at the bases of the pelvic spines, two soft rays in the pelvic fins instead of one or none, fewer gill rakers and dorsal and anal fin rays, a deeper body and shorter tail stalk (McAllister, 1960; Scott and Crossman, 1963). Second, the blackspotted stickleback is restricted to coastal waters from Newfoundland to Massachusetts (Leim and Scott, 1966). The threespine stickleback overlaps this range and in many

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areas along the New Brunswick coast bordering the Gulf of St. Lawrence the two species are abundant and apparently sympatric for much of their lives. The occurrence of morphologically similar sympatric species leads naturally to a consideration of reproductive isolating mechanisms.

MATERIALS AND METHODS

The study was made from May to August 1965 at a small field station located on the Miramichi River near Loggieville, New Brunswick, and during the summer of 1966 at University of Victoria, Victoria, British Columbia.

The fish used were captured with seines in the main river and smaller tributaries within a few miles of the station. In the laboratory the fish were held in tanks, plastic dish pans, and glass aquaria, with standing water whose salinity was adjusted with a commercial sea-salt mixture (Rila Products, Teaneck, N.J.) to roughly correspond to the salinities of natural breeding areas. In these areas salinity shows marked daily and monthly variation as a function of wind and tide, varying from fresh water to approximately 20‰ salinity (McKenzie, 1959).

For behavioural observations, glass-sided aquaria ranging in capacity from 3 to 50 gal were used, most observations being made in 20- and 50-gal tanks. To simulate the natural habitat as closely as possible each aquarium was provided with a sand bottom, a variety of algae and rooted aquatic plants, and one or more rocks or pieces of water-logged wood. Standing water of low salinity (approximately 12‰) was used and intermittently filtered through glass wool and charcoal. Natural photoperiods were maintained by means of large windows adjacent to the observation tanks. Aquarium temperatures varied with the season between 15 and 24 C. Habitat features which could not be duplicated were: the brightness of natural sunlight (in addition to natural light, single 20-w, cool white, fluorescent lights were suspended over each tank); changes in water level, temperature, salinity and water current due to the twice daily, and nearly equal, ebb and flood tides and the full natural community of plants and animals.

Most observations were made by watching individual fish for 30-min periods (on a few occasions 15-min observations were made and during spawning sequences observations sometimes lasted for 1.5 hr or more). Individual activities were described in detail and frequency and duration of selected activities were recorded, largely according to categories described by previous workers (van Iersel, 1963; Hall, MS, 1957; Sevenster, 1961; Symons, 1965; and Van Den Assem, 1967). Generally the fish were studied in community situations, that is, with other males and females of the species present and occasionally with other species. Besides *G. wheatlandi* mature specimens of four other locally available stickleback species (*Gasterosteus aculeatus*, *Apeltes quadracus*, *Calaea inconstans*, and *Pungitius pungitius*) were maintained for comparison. In particular attempts were made to hybridize *Gasterosteus wheatlandi* and *G. aculeatus*. Initially observations were taken from behind screens but this species adapts very readily to laboratory activities, unlike the brook stickleback, *C. inconstans*, for example.

Observations are grouped under the following headings: life history and nonreproductive activities, nest-building, territorial behaviour, courtship and spawning, parental behaviour, reproductive isolating mechanisms.

LIFE HISTORY AND NONREPRODUCTIVE ACTIVITIES

Mature fish appeared along the shores of the main river and at the mouths of smaller tributary streams in early May. Presumably they had migrated from coastal, perhaps ocean, waters since they were not available prior to this time in freshwater areas upstream to the point of capture. In the Miramichi River system sexually mature individuals were available from mid-May until mid-July. In comparison to other local species their reproductive period approximates that of the fourspine stickleback, *A. quadracus*, but begins several weeks later than those of the ninespine stickleback, *P. pungitius*, and the threespine stickleback, *G. aculeatus*.

Gasterosteus wheatlandi nested in brackish water of varying salinity depending on the tidal cycle, in creeks, streams, and smaller rivers tributary to the Miramichi. None were found to breed in freshwater areas where threespines and ninespines commonly nest. Instead nesting areas appeared to correspond more closely to those of *A. quadracus* (the fourspine stickleback), the mummichog (*Fundulus heteroclitus*), and the Atlantic silverside (*Menidia menidia*) in this region. Evidently the difference in nesting habitat between *G. wheatlandi* and *G. aculeatus* is a matter of behavioural selection since in both species the juveniles and adults were tolerant of a wide range of salinities.

My observations suggested that *G. wheatlandi* breeds during one season only. Post-spawning animals maintained in aquaria under a variety of salinities resumed feeding but survived only a few months. Also, in the Miramichi system mature blackspotted sticklebacks vary little in size, whereas three, four, and nine spines usually show at least two obvious year-classes. If any did survive, they presumably migrated seaward since no adults were seen in the river area from late summer until the following spring.

The following nonreproductive activities were characteristic of the species.

Swimming — In unhurried swimming the fish moved by a rowing action of the pectoral fins. The movements had an exaggerated, jerky character in comparison with those of the threespine stickleback possibly because the pectorals are larger. In rapid locomotion the caudal fin was also used. Turning was accomplished by differential use of the pectorals.

Stretching — In stretching, a variable number of fins and spines were raised deliberately, held stiffly erect for a few seconds, and then lowered simultaneously. This activity was sometimes combined with yawning and S-bending activities described below.

S-bending — The body was bent laterally into an "S" shape. Typically this posture was combined with stretching as described above.

Yawning — The mouth was opened for a fraction of a second usually with the dorsal and anal fins raised. The dorsal spines were partly raised as the mouth closed. Yawning sometimes occurred in association with the stretching and S-bending described above.

Chafing — The fish swam so that a particular part of the body was rubbed against chafing surfaces such as plants, sand, or rocks.

NEST BUILDING

Reproductive activities began with the selection of a nest area by the male. The following features were commonly found within the reproductive territories of wild males: a sandy or coarse mud substrate, abundant rooted aquatics, a solid vertical surface provided by stones or sunken logs, and thread-like plant debris and algae suitable for nest construction. Nests were built in open areas in depths varying from a few inches to about 2 ft at low tide.

In the laboratory the male began constructing the nest by digging a small pit. As digging proceeded he carried many pieces of plant material to the pit. The nest was shaped by a combination of additional activities: pushing served to mold the plant fragments, boring produced an entrance shaped to accommodate the males' head, glueing provided structural integrity, and sucking enabled sand to be removed from under the nest. As the nest neared completion the male occasionally tunnelled through it but promptly repaired the exit. Finally, he covered the nest surface with mouthfuls of sand so that it blended into the surroundings leaving the entrance as the only obvious feature.

Viewed from above the completed nest was elliptical in outline (approximately 1.5 × 2 inches) with a series of small sand-removal holes along the circumference. The entrance was located slightly off center on the long axis.

All nest building activities except tunnelling and sand covering were continued throughout the subsequent reproductive period. Two peaks were evident (Table 1): one immediately after spawning (nest repair) and a second on the day after hatching (nursery construction). Further details of nest building behaviours are given below.

Digging — Sand was removed from the nest site by biting into the bottom in a nearly vertical (90°) position. The mouthful of sand was carried away from the nest area, usually with dorsal and anal fins erect and often in a head-down position. Sand was released at least ½ inch above the bottom and the male carefully watched the grains of sand as they fell to the bottom, a behaviour which closely parallels that of egg-cleaning except for the absence of masticating movements. The pit which resulted from digging measured approximately 1 × 0.75 × 0.5 inch deep, the precise dimensions varying considerably from male to male and with the substrate. The frequencies of sand removal at various times are shown in Table 1.

Plant collection — During nest construction before spawning, in contrast to nursery construction, the male selected mostly unbranched plant materials varying from very fine strands of filamentous algae to pieces of rooted aquatics (roots and stems) several millimeters in thickness and from ¼ to about 2 inches long. Anchored strands were torn free by vigorous pulling movements. Materials were carried crosswise in the mouth, usually one piece at a time with dorsal and anal fins erect. This activity was typically followed by pushing and other nest-building activities described below. Towards the end of incubation additional plant materials, often large and branched masses, were carried or dragged to the nest. The nursery was thus a loosely organized mass of plant materials within which the newly hatched fry could be seen to swim and perch. The frequencies of plant collecting and removal are shown in Table 1.

Sand removal (*sucking*) — After considerable plant material had been collected, sand was removed from beneath the nest as follows. The male pushed his snout deep into the nest structure (Fig. 3A). This action was accompanied by exaggerated opercular pumping movements and quick swimming thrusts. Except for the foregoing, fin postures and body orientation were identical to those in digging. Sand removal was carried out regularly though not exclusively around the periphery of the nest resulting in a series of small holes ringing its circumference. Sand removal was commonest during nest construction when plant material made digging impractical, after spawning when most of the sand previously added to the nest surface had fallen into the nest, and finally during nursery construction. The frequencies of sand removal at various stages of the reproductive cycle are shown in Table 1.

Pushing — This activity served, along with glueing, to consolidate and shape the nest. Typically the dorsal and anal fins were raised and the push was delivered in a nearly vertical position (60–90°). Pushing was particularly common immediately after spawning during nest repair. Beyond this point it was repeated with decreasing frequency into the nursery phase of reproduction. Pulling was also seen occasionally. In this activity plant material was grasped in the mouth and gently pulled as if to loosen the nest structure. Occasionally plant materials were removed from the nest and discarded.

TABLE 1. Frequencies (average numbers of bouts in 30 min) of various male activities. Numerals in parentheses at the top of each column are numbers of observation periods.

Activity	Days before spawning[a] (3)	Day of spawning[b] (5)	Days after spawning 1 (7)	2 (5)	3 (4)	4 (4)	5 (3)	6–7 (5)	8–10 (5)
				Nest building					
Adding plants	0.3	9.0	1.9	1.8	2.5	1.5	1.7	2.4	2.0
Removing plants	0	0.8	0.6	0.4	0.3	0.8	1.0	1.0	0.8
Adding sand	0.7	2.5	1.4	1.0	0.8	1.3	1.7	0.8	0.0
Removing sand	1.3	1.0	2.7	4.0	1.5	0.5	2.3	0.8	1.0
Pushing	1.3	48.6	12.0	17.8	8.8	3.0	11.0	2.8	1.6
Boring	4.7	2.2	4.3	2.4	2.5	3.0	4.0	0.6	2.2
Glueing	2.7	10.6	5.9	1.0	0.3	0.0	4.7	0.0	0.0
Total	11.0	74.7	28.8	28.4	16.7	10.1	26.4	8.4	7.6
				Comfort movements					
Yawning	1.3	0	0	0	0	0	0	0.4	0.2
Stretching	0	0	0.1	0.6	0.5	0.2	0.4	0.8	0.8
S-bending	0.7	0	0	0	0.2	0	0.2	0.0	0.4
Chafing	0	0	0	0.6	0	0	0.8	1.4	0.8
Chewing	0	0	0	0	0.5	0	0	0	0
				Territorial behaviour					
Direct threat	–	7.8	13.4	4.2	5.0	2.8	1.3	2.0	2.6
Head-down threat	–	1.6	3.9	4.4	5.5	6.3	5.3	1.4	3.8
Broadside threat	–	0	0.3	0	0	0	0	0	0.8
Biting	–	11.0	12.3	4.0	3.8	2.0	2.0	1.8	1.2
Total		20.4	29.92	12.6	14.3	11.1	8.6	5.2	8.4
				Parental care					
Fanning	12.3	8.0	18.9	19.2	22.3	29.5	39.0	18.2	12.2
Avg length (*sec*)	(15.6)	8.0	12.6	11.9	19.3	15.9	12.6	10.1	8.8
Range (*sec*)	(7–37)	1–17	1–27	2–30	2–36	2–35	1–40	0–24	0–27
Vertical quivering	0	18.5	2.0	1.0	0	0	0	0	0
Fry retrieval	–	–	–	–	–	–	0.3	0.2	0.2

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[a] Combines observations from day 1 and 4 before spawning.
[b] Observations taken within 2 hr after spawning.

Boring — Boring in combination with pushing was used to shape the nest entrance. During boring the male inserted his snout into the nest entrance at an angle of approximately 45° with dorsal and anal fins erect. With repeated thrusts and lateral twisting the nest entrance was enlarged and deepened to accommodate the anterior one-third of the male's body. Each bout of boring lasted 6–8 sec. The frequency of boring varied considerably (Table 1) being commonest during nest construction, post-spawning nest repair, and nursery construction.

Glueing — The posture assumed in glueing was as follows: dorsal and anal fins stiffly erect, one or two dorsal spines usually partly raised, caudal fin and peduncle curved upward from the anal region. Application of the glue was accomplished by slowly swimming horizontally across the nest with the anal region closely applied to the nest surface and the whole body rapidly quivering. Each bout lasted approximately 6–8 sec. Insertion glueing, characteristic of ninespine and brook sticklebacks (Hall, MS, 1957), was not observed in this species.

Sand-covering — When the nest was substantially complete the male carried mouthfuls of sand back to the nest and with repeated trips covered the nest surface. In contrast to his habit in digging and sand removal the male did not watch the sand grains as they fell to the nest surface. If spawning was delayed some males removed and replaced the sand several times.

Tunnelling — Prior to spawning some males swam into the nest entrance and crept completely through the nest structure creating a tunnel with both entrance and exit. Among 24 nesting males this activity was observed only three times, each time with a different male. This contrasts sharply with a much higher frequency of tunnelling in *G. aculeatus*. In this species Nelson (1965) has suggested that tunnelling (creeping through) may play a causal role in motivation changes leading to a more sexual state. Sevenster-Bol (1962) tentatively viewed tunnelling as occurring through disinhibition resulting from a mutually inhibitory balance of sexual and aggressive drives. Neither interpretation seems applicable to *G. wheatlandi*. The low frequency of tunnelling, its timing (pre-spawning), and nonsexual context (in each instance no female was present) suggest that tunnelling may be more simply viewed as high intensity boring.

TERRITORIAL BEHAVIOUR

In the laboratory males tolerated the presence of conspecifics of both sexes during the early phases of nest construction. As construction advanced they became progressively more aggressive, initially to both sexes and later only to males and nonreceptive or spawned females. After fertilizing several clutches of eggs, the male again reacted aggressively to all adult conspecifics regardless of sex. Aggressive encounters between territorial males and male intruders were characterized by complex threats and fighting.

In nonsocial contexts the male's fins and spines with the exception of the pectorals were kept folded or partly depressed. Even the caudal remained partly folded except during vigorous swimming. None of the fins showed unusual colour patterns; all were a consistent pale and transparent white or yellowish colour (Fig. 3). Raising the two dorsal spines, the two pelvic spines, and the dorsal and anal fins appeared to be important especially in aggressive and sexual interactions.

Agonistic encounters typically began with the territorial male swimming directly and rapidly towards the intruder with dorsal spines and dorsal and anal fins raised (a direct threat), or in a head-down position displaying to a full advantage raised dorsal spines and fins (a head-down threat). These locomotory threats sometimes extended into a stationary threat with the territorial male broadside to the intruder and all fins and spines in the fully extended position (a broadside threat). Typically, bouts of biting ensued.

In the community aquarium situation biting was common in territorial encounters. Evidently bites were directed to any part of the opponent's body. One or sometimes more bouts of biting ended in the intruder escaping by swimming rapidly away from the territory though not necessarily out of visual range of the territory holder.

Further details of territorial behaviours follow.

Fin and spine raising — In aggressive encounters the dorsal and anal fins were usually raised. Of the two, the dorsal fin appeared to have the lower threshold. The spines consist of two dorsal and one pair of pelvic spines. Both had a higher threshold than the fins with dorsal spines having a lower threshold of stimulation than the pelvic. Occasionally the two dorsal spines were controlled independently. For example, during glueing one or two dorsal spines may be raised.

Direct threat — This was the commonest form of threat (Table 1). It was executed by the territory holder swimming directly toward the intruder. Fin and spine postures varied widely from a form in which: (1) no fins or spines were raised; (2) both dorsal and anal fins were extended; (3) dorsal and anal fins and dorsal spines were raised; and finally (4) all fins and spines were raised. Forms 2 and 3 were common, 1 and 4 rare. Other combinations were not observed. The variable form of direct threats showed certain regular features. For example, during the early stages of nest building threats often involved no fin or spine raising. The subjective impression gained was one of low intensity territorial interaction. During the later stages of nest building and during the parental phase direct threats were commonly performed with the dorsal and anal fins raised and in some instances the dorsal spines. The only occasion when the ventral spines were also raised was during the sexual phase possibly because they are also raised in the zig-zag dance and leading sequences. Spine and fin involvement, therefore, was to some extent correlated with other reproductive activities as Symons (1965) has shown for the threespine.

Head-down threat — This was the second commonest threat (Table 1). It was performed in a manner identical to that of the direct threat except that the territory holder swam towards the intruder in a head-down position (approximately 30–40° from the horizontal). Similar variability was observed with respect to fin and spine raising.

Broadside threat — In territorial encounters, usually involving biting, the territorial holder, between bites, sometimes assumed a position broadside to the intruder with all fins and spines raised. Spine fighting (van Iersel, 1953) was not observed.

The total frequency of all territorial behaviours is listed in Table 1. Agonistic behaviour in the community tank conditions of this study was maximal on the day of spawning and the day after probably in part due to the strong attraction that courtship and parental activities provided to non-nesting males.

COURTSHIP AND SPAWNING

With the nest more or less completed courtship began. When a female entered a nesting territory the male approached her aggressively like any other intruder. Nearing the female he apparently identified her visually by her greatly distended abdomen and characteristic posture. What began as a threatening approach by the male became the well known zig-zag dance, performed in a manner much like that of the familiar threespine. If receptive, the female assumed a head-up posture. The male sometimes interrupted the zig-zag dance to attack the female in a manner similar to aggressive encounters with males.

In a typical sequence the receptive posturing of the female stimulated the male to begin the leading sequence, a behaviour which differed sharply from that of the threespine stickleback. Briefly, the male adopted a head-down (30°) position on or very near the substrate (Fig. 3B). The dorsal and anal fins were raised, the dorsal spines partly raised, and the pelvic spines held stiffly erect. The body was bowed so that the ventral surface was concave and the male quivered rapidly. The female, maintaining the head-up posture, swam to a position beneath the male. With her mouth she nudged the male between his pelvic spines. The male then moved forward slowly, quivering rapidly and continuously. The female responded by swimming and pushing against the male. Typically, the pair followed a devious route to the nest entrance, the leading sequence lasting in one instance 2 min and 40 sec. An average time would be about 1 min. Uneven bottom in the nest area or rooted aquatics near the nest entrance often prolonged the leading sequence by interrupting tactile contact between the courting pair.

On reaching the nest the male showed the entrance as follows: he stopped, ceased to quiver but otherwise maintained the leading posture, inserted and withdrew his snout from the nest entrance several times in rapid succession, and then backed up and away from the entrance to allow the female a clear entry (Fig. 3C). The female entered the nest usually with considerable effort. Once in the nest only a few strands of algae and glue covered the female leaving most of the tail region exposed as well as the head (Fig. 3D).

The male stimulated the female to spawn (Fig. 3D) by moving along her posterior dorso-lateral surfaces, alternating irregularly from side to side, at the same time rapidly quivering and touching her with his mouth, an activity which continued until the female left the nest. After spawning the female retreated from the nest area in the normal swimming posture. In fertilizing the eggs the male simply swam through the nest tunnel without pausing. Detailed descriptions of courtship and spawning behaviours are given below.

Male approach — As he approached the female the male initially showed either a direct or head-down threat. Subsequent recognition of the female apparently depended mainly on visual cues. For example males courted females isolated either in floating glass jars or in adjacent aquaria suggesting that olfactory and auditory stimuli are not essential. Probably the most important visual cue was the female's distended abdomen. Spawned out females lacking this feature were attacked very much like intruding males even though the other cues which identified them as females appeared to be intact.

Zig-zag dance — Typically all fins and spines were carried in the fully extended position and the dance was performed in a head-down (30°) position. Each movement consisted of a rapid 1–2-inch dart toward the female followed by another dart on a course 30–90° to the first with successive changes in heading alternating to the right and left. The dance was sufficiently similar to that of the threespine that *G. aculeatus* females on some occasions were courted by and responded to *G. wheatlandi* males.

Receptive posture — Females, if receptive, responded to the zig-zag dance by assuming a head-up tail-down (approximately 30° from horizontal) position. As the male performed the zig-zag dance the female rotated to face the male head-on, often intensified the head-up posture, and swam toward the male.

Leading (*Fig. 3B*) — Typically the male initiated leading behaviour after a series of zig-zag dance and biting sequences. On rare occasions the zig-zag dance was omitted and the female immediately assumed a following posture to which the male responded by leading.

Following (*Fig. 3B*) — The female's posture in the leading sequence was identical to that of the receptive posture except with respect to her spatial relationship to the male. In following she swam to a position beneath the male so that her snout touched the male's ventral surface between his pelvic spines. Because the male led along the bottom the female's pelvic spines and abdominal region touched the substrate. In this position she appeared to fix visually on the male's stiffly erected pelvic spines. Once the two were in position continued forward motion seemed to require almost continuous contact. If the female stopped the male reversed his forward motion slowly backing up until he contacted her. If the leading sequence was interrupted frequently, the male usually attacked the female. Biting led to female escape, reversion to an earlier stage of the courtship sequence, or a resumption of leading. Leading was sometimes interrupted for territorial defence in which instance the female either rested on the bottom in the following posture or abandoned the courtship activities.

The combination of complex male and female postures and of continuous tactile contact, both during forward locomotion, made the leading–following sequence awkwardly intricate and particularly prone to accidental interruption. Most difficulties seemed to arise because it was carried out on the bottom. In one sense the bottom was useful because it served as a guide to both participants. However, any change in substrate topography, for example a vertical rise or fall of even ½ inch frequently interrupted tactile contact. Likewise, complex turns were almost impossible to execute. The problem was further complicated because the leading–following sequence followed a devious route to the nest entrance which sometimes required several minutes to traverse. Due to the length and complexity of the leading sequence the overall courtship behaviour had a much more deliberate and difficult quality than that of threespine sticklebacks. Although other parts of the courtship were sometimes abbreviated or eliminated the leading interaction apparently was essential to spawning.

Showing the nest entrance — The approach to the entrance was always made along the long axis of the nest starting at the end nearest the entrance. Leading continued until the male arrived immediately above the entrance. At this point he showed the entrance by inserting his snout and partially withdrawing it several times in rapid succession with all fins and spines raised.

Entrance of nest by female (*Fig. 3C*) — The female remained stationary during the showing sequence. When the male backed away from the entrance the female then attempted to enter the nest, simultaneously lowering her fins and spines. Getting into the nest always appeared to be a difficult task. The nests were always very compactly built structures, at least in the aquarium conditions prevailing in this study. In addition the distended abdomen of the female and the apparent failure of some males to tunnel through the nest required the female to literally fight her way in. This produced several additional complications. The courting sequence was frequently abandoned, usually temporarily, after several unsuccessful entrance attempts. In some instances she simply swam away. In others she remained in position over the entrance and the courtship sequence stalled. Males were never observed to bite females in the nest-entering position. Instead they remained rigidly in a terminal showing position, above and slightly behind the female with all fins and spines extended. The impasse was solved on some occasions by the female simply swimming away while the male was occupied with territorial defense. When the female finally succeeded in entering, the result was a thoroughly disrupted nest.

Spawning (*Fig. 3D*) — Once in the nest the male took up a position beside the female's tail region with dorsal and anal fins and usually dorsal spines extended. He then stimulated the female by quivering along her exposed dorso-lateral surfaces, alternating irregularly from side to side. Individual bouts of quivering lasted 3–6 sec. On the average 8.6 bouts (range 6–13) were required for spawning. The female remained for about 1 min, in an upright position in the nest. No obvious movements accompanied egg release.

Fertilization — Fertilization in some instances was delayed several minutes after egg deposition while the male chased intruders away.

The courtship sequence of *G. wheatlandi* differs from that of *G. aculeatus* in several respects. There are virtually no nest-building activities (such as glueing, fanning, or tunnelling) included in it. The male performs the zig-zag dance in a head-down rather than a horizontal position and thus an additional element, apparently agonistic in origin, has become ritualized as part of the dance. Leading, an unritualized activity in *G. aculeatus*, is highly ritualized in *G. wheatlandi*. Showing the nest entrance and spawning activities are very similar except that no evidence of "heeling over" or of "pricking" (Hall, MS, 1975) was observed with *G. wheatlandi*.

Nest building and parental activities were resumed or begun at various intervals (min) after fertilization as follows:

	Plant collecting	Sand removal	Pushing	Boring	Glueing	Fanning	Vertical quivering
Avg	17.3	8.5	2.3	4	7.5	47.5	14.3
Range	8–30	8–9	2–3	–	4–10	46–49	12–17
No. of observations	3	2	4	1	4	2	4

In general these intervals parallel rather closely those given for *Gasterosteus aculeatus*. (Hall, MS, 1957).

PARENTAL BEHAVIOUR

The immediate post-spawning activities were concerned mainly with nest repair. If, as often happened, the female displaced some eggs from the nest when she left, the male retrieved and replaced them through the entrance. He then employed all nest-building behaviours except sand collection and tunnelling to restore the nest to its pre-spawning condition.

A distinctive post-spawning behaviour of *G. wheatlandi* was provisionally called *vertical quivering*. What purpose it serves is not clear. The male assumed a vertical position (90°) with the head inserted deeply into the nest at or near the entrance, and quivered in short bursts of 1–6 sec. There were no opercular movements as in sand removal. The activity was most common in the 30-min period after spawning and appeared to be gradually replaced by fanning (Fig. 1).

Once the nest was repaired, fanning (Fig. 3E and G) occupied much of the male's time and closely resembled fanning in the threespine stickleback. Egg cleaning was also observed on a few occasions. The male carried eggs away from the nest, usually several at a time, gently masticated them, and spat them out. As they slowly sank toward the bottom he again caught them. The process of mastication, spitting, and catching was usually repeated several times with each group of eggs. He then either discarded them or replaced them in the nest.

Towards the end of incubation the male added, with little or no pushing, substantial quantities of plant material to the nest surface. This had the effect of providing a loose mass of plant material within which the newly hatched fry readily could move. During the nursery phase if fry in the early free-swimming stages moved away from the nest structure the male caught them, carried them back, and forcibly injected them into the nest. Detailed descriptions of parental behaviours including egg stealing are given below.

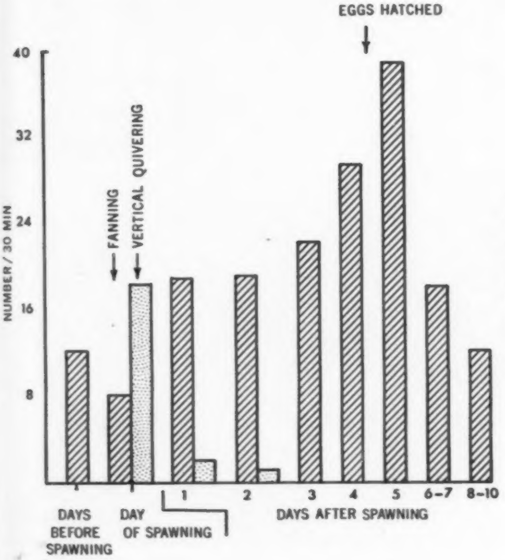

FIG. 1. Fanning and vertical quivering (mean bout frequencies) at various stages of the reproductive cycle. Pre-spawning combines three sets of observations from days 1 and 4 before spawning. Spawning day observations were made during the 2-hr period immediately after spawning.

Fanning (*Fig. 3E and G*) — The male assumed a position 0.25–0.5 inch above the nest entrance at an angle of approximately 45° from the horizontal with both dorsal and anal fins erect and the tail stalk bent slightly dorsally. Rapid beating of the pectorals created a current of water directed into the nest entrance while compensatory movements of the caudal fin enabled the male to remain stationary. The intensity of fanning varied considerably. Rapid pectoral beats produced water currents strong enough to displace loose nest materials. From this high intensity, fanning decreased to a point where pectoral movements were slow or completely absent. For example after the young had hatched the male sometimes assumed the fanning posture at the nest entrance but omitted any pectoral movement (Table 1, days 6–10, range data).

Fanning was a common activity during all stages of reproduction in the blackspotted stickleback. It began during nest construction, increased in frequency and intensity during egg incubation, and decreased after hatching. Figure 1 shows that fanning reached a minimum value immediately after spawning. During this period another activity, vertical quivering, dominated behaviour at the nest entrance. In general the average duration of fanning bouts (Fig. 1) increased with increasing bout frequency. However, as Morris (1958) noted for the ninespine, *P. pungitius*, and the threespine, *G. aculeatus*, the peak in bout duration precedes that of bout frequency. Hatching occurred between the two peaks.

The preceding observations closely parallel those of van Iersel (1953) for the threespine stickleback with one notable exception. Whereas *G. aculeatus* in van Iersel's study fanned for periods in excess of 1000 sec per 30-min observation period, the maximum figure for *G. wheatlandi* was never more than 500 sec, a value which compares favourably with Morris' (1958) observations of 400 sec per 30 min for the ninespine stickleback. van Iersel (1953), Hall (MS, 1957), and Morris

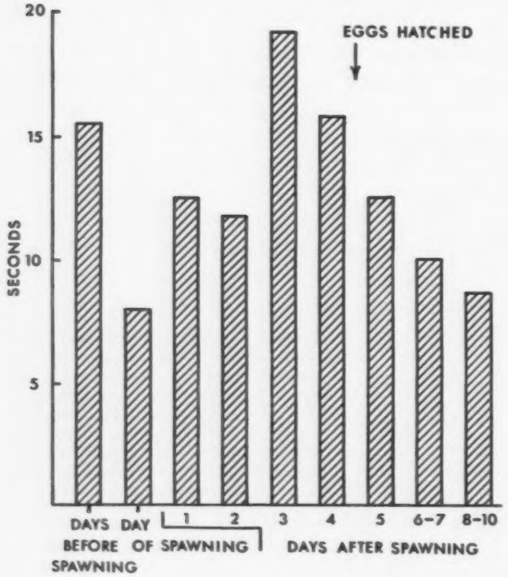

FIG. 2. Fanning bout lengths at various stages of the reproductive cycle. Refer to Fig. 1 for abscissa details.

(1958) reasoned that the greater fanning rate of the threespine may be due to the poorer environmental situation in ground nests as compared with the elevated nests of other species and to the larger eggs of threespines (1.44–1.52 mm) (Van Den Assem, 1967). In spite of both ground nests and large eggs (1.2–1.5 mm) (Scott and Crossman, 1964), *G. wheatlandi* shows low fanning rates so that some other explanation is necessary. Possibly the efficiency of ventilation differs between species. Until suitable comparisons of fanning efficiency are made a conclusion is not possible.

Vertical quivering — This activity of unknown function is performed in a vertical position (90°) in or immediately adjacent to the nest entrance. The dorsal and anal fins are raised and occasionally the dorsal spines. As the male's snout is thrust deeply into the nest the body quivers rapidly for 1–6 sec (average 3.9 sec). There are no opercular pumping movements as in sand removal. The highest frequencies for vertical quivering occur immediately after spawning (Table 1, Fig. 1) and thereafter decrease rapidly as fanning frequency increases. Two functions suggest themselves. Vertical quivering may aid fertilization by mixing eggs and sperm or this activity may be analogous to vertical boring (boring fanning) in the threespine stickleback. According to van Iersel (1953) this activity occurs a few minutes after spawning and possibly serves to fasten the eggs in the nest cavity. The quivering component appears to be unique to *G. wheatlandi*.

Egg retrieval — Though relatively uncommon there were several situations in which males manipulated eggs outside the nest structure. If after spawning some eggs were left outside the nest, the male replaced them in the nest tunnel. During the course of incubation eggs were sometimes removed from the nest entrance and cleaned as described above. Males in the parental phase of reproduction were never observed to eat their own eggs. They were either abandoned or restored to the nest. As described below eggs may be handled during egg stealing and either eaten or deposited in the thief's nest. Eggs artificially introduced into the nest area were eaten in the present study.

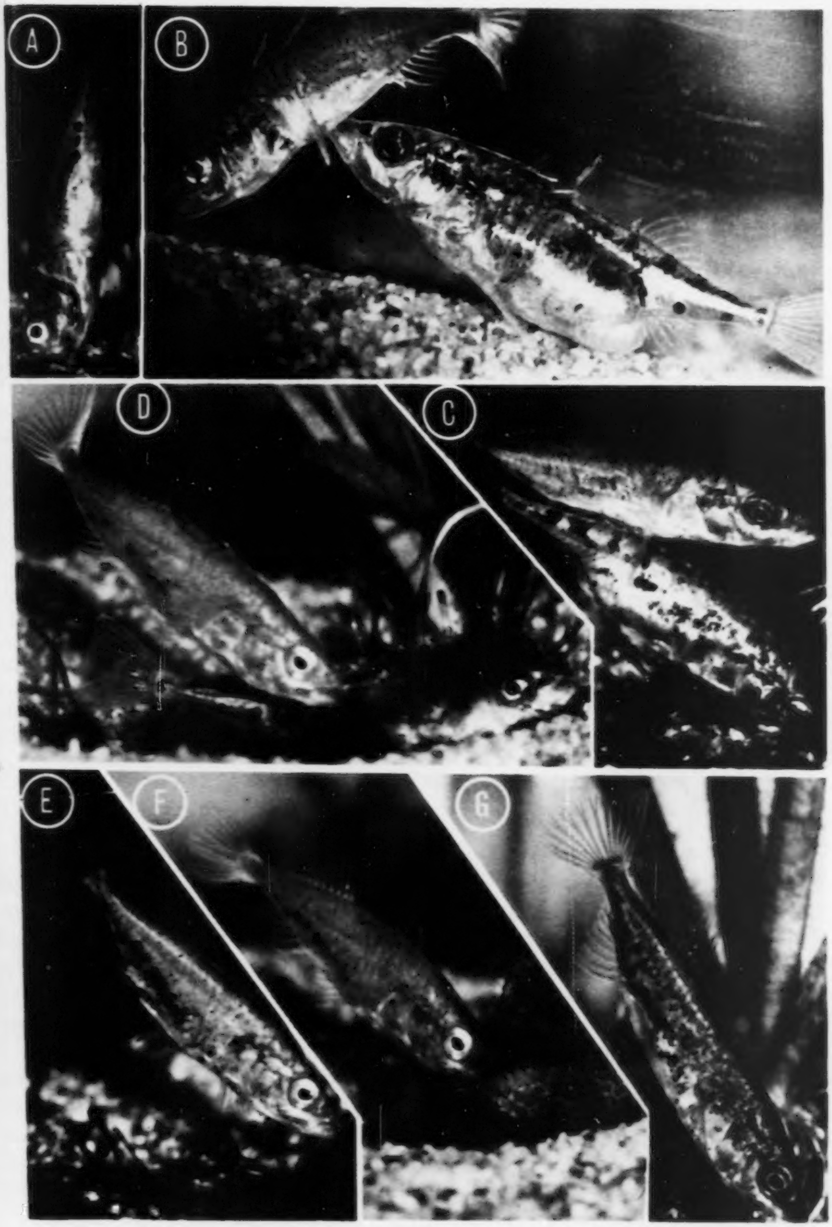

FIG. 3. Reproductive activities of the twospine stickleback *Gasterosteus wheatlandi*: (A) male removing sand (sucking — note opercular pumping action) with nest partly sand covered; (B) male and female in leading-following postures (note female's visual fix on male's pelvic spines); (C) female entering nest, male in terminal showing posture; (D) female in spawning position, male quivering along her side; (E) male in characteristic spawning colouration, fanning at nest entrance; (F) male arranging egg-mass immediately after spawning, just before repairing nest; (G) male in fanning position after eggs have hatched (note cryptic colouration at this stage as compared with that of same male in C).

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Fry retrieval — In the parental phase of reproduction young which wandered out of the nursery structure were caught by the male and returned to the nest. Keeping track of 50 or more young leaving the nest in all directions soon became an impossible task.

Egg stealing — This is a male activity observed in community tank (50 gal) situations where several nonnesting males (3–6) were present and territorial encounters were common. It is possible that there was not enough bottom area to accommodate more than two or three nesting males and that the tank was, therefore, unnaturally crowded. Egg stealing is not unique to blackspotted sticklebacks. It has been studied by Van Den Assem (1967) in threespines and by Morris (1952) in ninespines. Although restricted the present observations agree substantially with those of Van Den Assem for the threespine. Both pre-spawning nesting and nonnesting males were observed to steal eggs. Stealing began with the intruding male sneaking along the bottom towards the nest to be plundered. During the sneaking activity the male assumed a cryptic colouration and moved toward the nest in slow steps, a fraction of an inch at a time. At the same time the nest owner was leading a female to his nest, showing the nest entrance, or stimulating the female to spawn. These activities in particular seemed to exert a strong attraction to any males within visual range of the courting pair except those in the parental stage. After sneaking to a position several fish lengths from the nest entrance, the plunderer suddenly dashed toward the nest entrance with all fins and spines raised. If this occurred prior to spawning the nest owner drove the intruder away. The only instances of successful stealing were observed as the female left the nest after spawning and before the nest owner had fertilized the eggs. In this situation the intruder fertilized the clutch, removed a mouthful of eggs, and carried them off while the nest owner was distracted with other territorial encounters. The fate of the stolen eggs varied. If the thief was a nonnesting male the eggs were apparently eaten, sometimes after being carried around for several minutes. If the thief was a nesting male the eggs were carried back to his nest. In one instance the thief was in the early stages of nest construction and he literally built the nest around the stolen eggs. This particular male made several trips between the two nests each time returning with a mouthful of eggs. He appeared to be able to enter the plundered nest with little opposition from the owner. In one instance several males participated in plundering a nest with ineffectual opposition from the owner. In this instance also the eggs were fertilized by an intruder.

REPRODUCTIVE ISOLATING MECHANISMS

Three major factors probably contribute to reproductive isolation. First, field observations indicated that *G. wheatlandi* breeds only in brackish water and *G. aculeatus* in fresh water. Even with such geographic separation *G. aculeatus* females may breed with *G. wheatlandi* males as the former migrate through estuarine areas into fresh water. Also, the upstream segments of *G. wheatlandi* populations may overlap with downstream portions of *G. aculeatus* breeding populations.

A second factor depends on female selection of species-specific male colour patterns. In contrast to the red and blue highlights of *G. aculeatus*, *G. wheatlandi* males have an overall greenish-gold colour with a series of black markings ventrolaterally behind the pelvic spines. The development of the black spots varies greatly between individuals (Fig. 3). After spawning *G. wheatlandi* males become more cryptically coloured (Fig. 3C and G).

Aquarium observations where mature males and females of both species were paired in all combinations of species and sex showed that *Gasterosteus* males did not distinguish between females. Both species of males courted both species of female equally vigorously. This was especially evident where the courting pairs were limited to visual contact through the glass walls of adjacent aquaria or where females were presented to a courting male in floating glass beakers. In contrast females clearly recognized and responded to male colours of their own species. This was best observed where receptive females of both species were individually introduced directly into the territories of nesting males of each species. In spite of similar male courtship activities (zig-zag dancing and biting) females in mixed species pairs, with few exceptions, showed only a temporary interest in the male and then swam away. Persistent male courting did not change this response.

Several noteworthy exceptions to male colour selection by females were observed. In each instance the female was very mature as indicated by extreme abdominal distension and apparently "highly motivated" sexually. In three instances when *G. wheatlandi* females were paired with *G. aculeatus* males the courtship sequence developed normally up to the leading behaviour. At this point the *aculeatus* male simply swam to the nest entrance leaving the female in a head-up posture on the substrate. None of the females showed any overt interest in the nest even when the male and nest entrance were in sight. In one instance when a *G. wheatlandi* male was paired with a *G. aculeatus* female the courtship sequence stalled with the male in his leading posture, head-down and quivering on the substrate and the *G. aculeatus* female showing a head-up posture in mid-water. The leading–following interactions are therefore incompatible and constitute a third isolating mechanism.

Presumably in the natural environment breeding habitat separation and male colour selection by females would effectively prevent hybridization. If these failed, a third mechanism, that of species-specific and incompatible leading behaviours, would virtually guarantee reproductive isolation.

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Buoyancy of Atlantic and Pacific Herring[1,2]

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Pacific and Atlantic herring after adjustment to water 36 cm deep had sinking factors between 1000 and 1008 and showed an inverse relationship between oil content and swimbladder volume up to 12% oil. At higher oil contents a swimbladder volume between 2.6 and 3.0% of total body volume was maintained. The mean volumes and densities of various components of the Pacific herring held in captivity were: swimbladder gas 4.1% of total volume, .0013 g/ml; oil 3.5%, .926 g/ml; scales 0.5%, 1.966 g/ml; skeleton 1.2%, 1.993 g/ml; rest of fish 90.6%, 1.057 g/ml. These components on the average exerted upward forces of 41.4 and 3.3 dynes/ml of fish due to gas and oil, and downward forces of 4.6, 11.2, and 32.1 dynes/ml due to scales, skeleton, and the rest of the fish respectively. Under natural conditions herring usually have high oil contents so the relationship observed here suggests they have low swimbladder volumes. This combined with a duct direct from the swimbladder to the exterior and the lack of gas secretion would give the herring freedom of vertical movement and a low change of sinking factor with depth.

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INTRODUCTION

MARINE FISH, although in a better position with respect to buoyancy than freshwater fish, still have body tissues which are denser than sea water. Jones (1951) calculated that free-swimming marine teleosts could decrease the density of their body to approximately that of the sea by having a swimbladder occupying about 5% of their volume. As fish descend, the increasing pressure decreases the volume of the swimbladder, and more gas is secreted into the swimbladder in all physoclists and many physostomes. An upward movement similarly is accompanied by the removal of gas from the swimbladder by absorption or gas release through the pneumatic duct. These changes in the mass of gas present enable the fish to compensate for compression or rarefaction of the gas in a direction tending to restore approximate neutral buoyancy.

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The herring differs in many ways from these typical marine fish. First, herring usually have swimbladder volumes below 5% (Brawn, 1962). Secondly, neither Atlantic nor Pacific herring are able to add gas to the swimbladder by secretion (Brawn, 1962, MS, 1964). Thirdly, herring are capable of storing in their bodies large amounts of oil, which contribute to their buoyancy but which, unlike the swimbladder gas, cannot be rapidly adjusted. Fourthly, the herring has a duct from the swimbladder to the exterior through which gas may be lost during decompression (Brawn, 1962). In spite of these differences the herring is not confined to the surface but undergoes regular diurnal vertical migrations and may descend to depths of 500 m by day (Devold, 1963).

As herring are incapable of adding gas to the swimbladder by secretion but rely on air taken from the surface to increase gas content, it should be possible to calculate herring density at any depth providing the density of the surface dwelling fish and its swimbladder volume are known. If the amounts and densities of each of its major components are also known, the contribution each component makes to the buoyancy of the herring can be determined. For this reason Atlantic herring, *Clupea harengus* L., from Passamaquoddy Bay, N.B., and Pacific herring, *Clupea pallasii* Valenciennes, from Howe Sound, B.C., were tested while still alive for swimbladder volume and total density and for volume and density of some other components after death.

METHODS

Swimbladder volumes were determined for living herring adjusted for at least 2 days to a depth of 36 cm using the method of Alexander (1959a) and the apparatus described by Brawn (1962). In this method changes in swimbladder volume are measured as movements of a menicus along a capillary tube, the apparatus permitting a resolution of .0013 ml. After recording the changes in swimbladder volume with increases in applied pressure up to 18 cm Hg the pressure was slowly reduced below atmospheric pressure to determine the pressure and change in swimbladder volume at which the fish attained neutral buoyancy. From the neutral buoyancy data and the density of sea water the density and volume of the fish at test depth were calculated. After the determination the herring were weighed and frozen for future analysis. Atlantic herring were analysed only for oil content but the volume and density of scales, skeleton, oil, and "the rest" were determined for Pacific herring.

To determine the oil content of individual Atlantic herring the frozen fish was sliced, and ground with three parts of anhydrous Na_2SO_4 to one part of herring by weight, and the oil extracted from the resulting powder at room temperature by three changes of ethyl ether during 3 hr. The filtered ether extract was evaporated and the oil dried to constant weight at 50 C.

The more complete analysis of the Pacific herring necessitated a more complex method. The frozen fish, wrapped in filter paper in a test tube, was heated in a steam bath for 7–15 min and dissected to remove skeleton and scales with skin. These were placed in separate test tubes and their oil extracted with ether. The remaining parts were ground with anhydrous Na_2SO_4 and, together with the filter papers which surrounded the fish during the steaming and dissection, were extracted with ether allowing at least 150 ml of ether for each gram of oil extracted and two or three changes of ether. The ether extracts from all fractions were filtered into a common flask and the total ether weighed. From the oil contained in two 25-ml samples of ether from the flask the total oil present in each fish was calculated. Oil extracted from all Pacific herring was pooled, cooled to 0 C and its density determined, while slowly warming from 8 to 20 C, by the weight thermometer method. The initial cooling was to ensure that the oil was solidified as it seems likely that the oil is in this form at the temperatures below 15 C at which herring live (Bailey, 1952).

To test the efficiency of the oil extraction 2 g of oil were added to the residue of extractions for the equivalent of a 100-g herring and the material extracted again. The estimated oil content differed by 0.11 g from the known weight, or by 0.12% of the volume of the fish.

After oil extraction the scales and skeleton were rinsed with distilled water, soaked 24 hr in 6% hydrogen peroxide, rinsed, and placed in distilled water. Adhering flesh or skin was removed and the clean scales and skeleton in water were placed in a vacuum oven to remove gas bubbles. The weight of the scales and skeleton in distilled water at known temperature was determined, and their constant dry weight. From weight in water and in air the density and volume of scales and skeleton were calculated using Archimedes' Principle. By the same method one determination of the density and volume of the testes and one of the ovaries in their normal wet state were made.

All volumes were expressed as percentages of the total volume of the fish, and the densities, when these varied with temperature as did oil, are given for the temperature at which the living fish was tested. The density and volume of "the rest" were found by subtraction of the skeletal, scale, oil, and swimbladder values from the density and volume of the living fish. The density of swimbladder gas was assumed to be that of air at the test temperature.

BUOYANCY OF ATLANTIC AND PACIFIC HERRING

The live herring of both groups had densities close to the density of the sea water in which they were held and so their sinking factors, the density of the fish divided by the density of their environment × 1000 (Lowndes, 1942), were all close to 1000 (Tables 1 and 2). Since herring adjust their density by liberating gas from the swimbladder or swallowing air at the surface to attain a low sinking factor (Brawn, 1962), the difference in density shown by the Atlantic and Pacific herring can be attributed entirely to the higher salinity (32–33‰) and hence greater density of the sea water in which the Atlantic herring were tested compared with the salinity (25–30‰) used for Pacific fish.

MAJOR COMPONENTS OF PACIFIC HERRING

Of the components thought most likely to affect buoyancy in the Pacific herring, the lowest in density was swimbladder gas (Table 2). The swimbladder volumes, expressed as percentages of the volumes of the fish, varied from 5.4 to 2.6% showing that the herring can adjust its swimbladder volume considerably to alter body density in a direction resulting in a low sinking factor.

The ether-extracted oil of Pacific herring had a density of 0.931 g/ml at 8 C and decreased by .001 g/ml for each degree rise in temperature up to 20 C. At the temperatures close to 13 C at which the test fish were living the oil had a mean density of 0.926 g/ml. Thus herring oil resembles the four teleost oils with a mean specific gravity of 0.91 given by Spector (1956) or cod liver oil of specific gravity 0.93 (Denton, 1963). It is unlike the oil of some sharks which is modified by the inclusion of squalene to have an unusually low density (Denton, 1963). Oil in the Pacific herring tested occupied from 12 to less than 1% of the total volume. The lowest oil contents are not typical of Pacific herring but result from the period the fish were held in captivity before testing. Pacific herring caught at monthly intervals for a year and

TABLE 1. Densities (ρ), oil and swimbladder volumes, and sinking factors of living immature Atlantic herring adjusted to 36 cm water depth. The density of swimbladder gas was taken as .0013 g/ml, and that of oil at the temperature of the tests was .926 g/ml.

Fish	Days at 36 cm depth	Pressure reduction to attain neutral buoyancy (*cm Hg*)	Fish		Swimbladder vol (%)	Oil vol (%)	Seawater ρ (*g/ml*)	Sinking factor
			Vol (*ml*)	ρ (*g/ml*)				
				Thin fish				
A 1	34	1.2	11.289	1.0295	5.23	0.7	1.0247	1005
2	34	2.0	13.930	1.0256	3.09	0.5	1.0243	1001
3	34	4.6	11.258	1.0286	4.72	0.6	1.0242	1004
4	34	4.0	8.754	1.0266	4.82	1.8	1.0240	1003
5	35	4.5	15.983	1.0279	4.73	0.7	1.0238	1004
6	35	3.2	22.484	1.0263	4.67	0.8	1.0240	1002
				Fat fish				
7	2	<14.0	36.476	>1.0307[a]	2.65	11.0	1.0240	>1007
8	2	<15.0	29.567	>1.0317[a]	3.04	16.6	1.0238	>1008
9	3	–	29.072	–[a]	3.93	6.6	1.0243	–
10	3	12.5	25.573	1.0330	4.01	7.3	1.0246	1008
11	3	10.0	23.933	1.0308	3.04	7.1	1.0245	1006
12	3	< 4.0	28.268	>1.0262[a]	3.02	9.9	1.0242	–
Means								
Thin		3.2		1.0274	4.54	0.9	1.0241	1003
Fat		11.2		1.0319	3.28	9.8	1.0242	1007
All fish		7.0		1.0285	3.91	5.3	1.0242	1005

[a]Density of these fish was taken as 1.0319 g/ml.

TABLE 2. Total densities (ρ), densities and volumes of various body components, and sinking factors of living Pacific herring adjusted to 36 cm water depth. The density of swimbladder gas was taken as .0013 g/ml.

Fish	Days at 36 cm depth	Pressure reduction to attain neutral buoyancy (*cm Hg*)	Fish		Swim-bladder vol (%)	Oil		Scales		Skeleton		"The rest"		Sea-water ρ (*g/ml*)	Sinking factor
			Vol (*ml*)	ρ (*g/ml*)		Vol (%)	ρ (*g/ml*)	Vol (%)	ρ (*g/ml*)	Vol (%)	ρ (*g/ml*)	Vol (%)	ρ (*g/ml*)		
Mature male															
P 1	25	12.2	33.717	1.0268	2.58	12.2	.926	0.2[a]	2.263	1.0	1.917	84.0	1.060	1.0213	1005
2	10	4.8	51.370	1.0245	4.63	2.5	.928	0.5	1.842	0.9	1.997	91.5	1.065	1.0213	1003
3	12	4.6	22.416	1.0249	4.34	2.5	.927	0.6	1.866	1.1	2.235	91.4	1.056	1.0216	1003
4	5	2.8	14.792	1.0213	4.96	1.3	.926	0.8	1.922	1.6	1.882	91.2	1.055	1.0193	1002
Mature females															
P 5	31	6.1	17.694	1.0265	4.20	1.3	.925	0.6	1.968	1.4	2.126	92.5	1.052	1.0216	1005
6	28	–	25.125	[b]	2.72	8.3	.926	0.4	1.857	1.3	1.647	87.3	1.055	1.0213	–
Immature															
P 7	3	2.0	7.755	1.0230	3.65	0.4	.928	0.4	1.820	1.2	1.866	94.3	1.049	1.0216	1001
8	19	6.0	13.863	1.0257	4.32	3.3	.924	0.6	2.023	1.2	2.153	90.6	1.057	1.0216	1004
9	11	3.8	28.292	1.0246	4.49	0.6	.927	0.4	1.911	1.1	2.045	93.4	1.058	1.0213	1003
10	31	5.6	16.681	1.0248	3.87	5.7	.925	0.4	1.974	1.0	2.265	89.0	1.056	1.0216	1003
11	7	3.7	20.646	1.0215	5.43	0.8	.925	0.6	1.922	1.5	1.933	91.6	1.062	1.0188	1003
Spent															
P12	4	7.5	50.458	1.0258	3.74	5.2	.923	0.3	2.348	1.1	2.000	89.6	1.058	1.0220	1004
13	5	3.2	27.167	1.0216	4.91	1.6	.926	0.6	1.840	1.2	1.842	91.7	1.061	1.0193	1002
Mean, all fish		6.0		1.0239	4.14	3.5	.926	0.5	1.966	1.2	1.993	90.6	1.057	1.0210	1003

[a] Some scales may have been lost from P1.

[b] No neutral buoyancy was obtained for this fish. Its density was assumed to be 1.0265 g/ml.

analysed by McBride et al. (1959) contained from 4.9 to 24.4% oil by weight and better represent the normal composition of the species.

The scales and skeleton, with mean densities of 1.966 and 1.993 g/ml respectively, had the highest densities of the components analysed. These components have been reduced so that scales occupy on the average only 0.5 and the skeleton 1.2% of the volume of the fish. This reduction is evident when the total bony content of 1.7% in herring is compared with values (expressed on a volume-per-100-g basis, which approximates percentage volume as the fish density is close to 1) given by Alexander (1959b): 4.3 for roach, 2.8 for dace, and 4.4 for carp. In these fish the density of scales and skeleton in the same species was similar and the herring resembles them in this respect. The mean density of 1.97 g/ml for the bony structures of herring was close to the specific gravity of bone of 1.9 for roach, 2.0 for dace, and 1.6 for carp. Thus herring reduce the downwards force due to scales and skeleton by reducing the amount of this material present rather than by decreasing its density.

Subtracting the total volume and mass of the components mentioned above from the volume and mass of the entire fish showed that "the rest" averaged 90% of the volume of the fish. Its density showed little variation round the mean of 1.057 g/ml.

Among components lifting Pacific herring in the water the force due to the swimbladder gas contributes more to the buoyancy of the fish than the upward force due to the oil even when the oil content reaches 12% (Table 3). At some depth below the surface the swimbladder gas would be so compressed that oil would replace it as the most important lifting component. Of components which exert a force downward the most important is "the rest." The scales and skeleton together exert a downward force equal to about one-third the force due to "the rest" although their respective volumes are 1:63. This emphasizes the importance of skeletal reduction in reducing the downward force on the fish.

The downward forces exerted by scales, skeleton, and "the rest" appear to vary independently of the amount of oil present and give a mean downward force of 47.9 dynes/ml of fish. The upward forces complement each other as an increase in buoyancy due to oil is compensated for by a decrease in the upward force due to the swimbladder gas. At the two highest oil contents overcompensation occurs and the force due to the swimbladder gas decreases more than the force due to the oil increases. As a result the total upward force is reduced and the compensating force which the fish must exert on the water if it is not to sink increases from a mean of 3 dynes/ml to 5 dynes/ml.

In Table 2, in which the percentages and densities of the components of Pacific herring were grouped according to the sexual condition of the fish, no consistent differences were evident between the mature male or female and the immature or spent fish. The effect of gonads on the forces acting on the fish can be calculated for fish P2, which contained testes occupying 10.8% of its volume and of density 1.0674 g/ml. Of the downward force of 3932

TABLE 3. Forces in dynes acting on a fish, on the basis of a 100-ml volume, at 36 cm depth due to various components.[a] The forces acting on Pacific herring were calculated from the data of Table 2; those on a roach in fresh water, from information given by Alexander (1959b); those for a single pollock, in the same manner as for the herring (Brawn, 1965). The small scales of the pollock could not be isolated and so are included in "the rest."

Fish	% oil	Swim-bladder up	Oil up	Scales down	Skeleton down	"The rest" down	Total		Compen-sation needed up
							Up	Down	
				Pacific herring					
P 7	0.45	3653	41	321	977	2535	3695	3833	138
P 9	0.56	4493	52	384	1084	3391	4545	4860	315
P11	0.85	5420	78	549	1338	3864	5498	5750	252
P 4	1.31	4953	120	744	1312	3214	5073	5270	197
P 5	1.31	4204	124	585	1484	2749	4328	4818	490
P13	1.56	4903	142	507	1001	3759	5046	5267	221
P 3	2.51	4344	233	522	1298	3130	4577	4950	373
P 2	2.50	4633	234	370	861	3932	4867	5164	297
P 8	3.26	4324	311	570	1332	3130	4635	5032	397
P12	5.25	3745	508	429	1046	3146	4253	4622	369
P10	5.68	3873	540	420	1268	3037	4414	4726	312
P 6	8.32	2722	778	344	774	2902	3499	4020	521
P 1	12.24	2582	1144	231	834	3191	3726	4256	530
Mean, herring		4143	331	460	1124	3206	4474	4790	316
				Other species[b]					
Mean, roach		9700	70	1801	1872	5806	9770	9479	291
Pollock		4097	98	–	1626	2623	4195	4249	54

[a] To find the true force in dynes acting on a fish the values given should be multiplied by $\frac{\text{volume of fish, ml}}{100}$.

[b] The mean sinking factors and swimbladder volumes for the three species were:

	Pacific herring	Roach	Pollock
Sinking factor	1003	1003	1002
Swimbladder volume	4.1%	9.9%	4.1%

dynes/100 ml due to "the rest" calculated for this fish 488 dynes/100 ml were due to the testes. The volume of the ovaries was not determined for any of the fish of Table 3 but if we assume they occupy the same volume as the testes and have the density determined here of 1.1022 g/ml the ripe female P5 would experience a downward force of 854 dynes/100 ml due to the ovary out of a total of 2749 dynes for "the rest." The downward force due to "the rest" was not consistently greater in mature than immature fish.

The comparison of the forces acting on the herring due to its various components with those acting on other fish is handicapped by the scarcity of comparable data. Alexander (1959b) gives data for the roach which were recalculated to give the forces acting on the fish in fresh water (Table 3). In the roach the upward force due to oil is small compared with that of the herring and the swimbladder contributes most of the lift to the fish. The bony structures exert a greater downward force on the roach than on the herring, due in part to the supporting medium being fresh water instead of sea water but mainly to the reduction of the bony parts, especially the scales, in herring. The sum of all these forces leaves a compensating force to be exerted by the fish of about 3 dynes/ml for both fish.

The pollock (*Pollachius virens*) from the Atlantic coast gives values roughly comparable with those for herring. It relies less on oil as an upward force than does the herring but gets similar support from the swimbladder. The skeleton of the pollock contributes a downward force greater than that of the herring. The lower sum of upward forces and the increased downward force due to the skeleton in the pollock are balanced by a lower downward force due to "the rest" (even though this included minute scales) so that the pollock has to exert less force to compensate than the herring.

It is possible to calculate the contribution water makes to the buoyancy of herring although in the analysis here it is included in "the rest." Iles and Wood (1965) have shown the relationship, in terms of per cent weight, between oil and water content for whole Atlantic herring minus gonads and gut contents. Using the densities determined here and the swimbladder volume corresponding to the oil content the relationship between water and oil may be expressed as per cent volume. Such a conversion shows that an Atlantic herring with no oil contains 81.5% water by volume. As the oil content increases the water content decreases so that a herring with 10% oil contains 74% water. In sea water of 1.021 g/ml the loss of water associated with this increase in oil in a 100-ml fish would cause a loss of uplift of 206 dynes but there would be a gain of 934 dynes from the oil for a net gain of 728 dynes acting upward. In a living herring at 36 cm depth the decrease in swimbladder volume which occurs more than compensates for this increased buoyancy so that the total upward force does not increase (see Table 3). As stated by Iles and Wood the observed oil/water relationship is part of the process by which herring can accumulate large amounts of oil without becoming too buoyant. The other adjustment, as shown below, is the reduction in swimbladder volume which, in the example given here, is of far greater significance than water loss since it would decrease the upward force by 2100 dynes compared with 206 dynes from water loss.

RELATIONSHIP BETWEEN OIL AND SWIMBLADDER VOLUME

The herring differs from the species with which it was compared above in its great range of oil content: from under 1% in some of the fish tested here to 25 or 30% in the fattest wild fish (Wood, 1958; McBride et al., 1959). A changing oil content obviously affects the buoyancy of the fish and since, as shown here, herring with less than 1% oil are almost in neutral buoyancy the accumulation of oil would make herring positively buoyant if there were not some compensatory mechanism. Iles and Wood (1965) showed that some decrease in water content occurs as oil content increases which would increase somewhat the density of fatter fish. These authors suggest that there may be a limit to the extent of swimbladder volume reduction as the swimbladder probably has a sensory as well as a hydrostatic function. The Atlantic and Pacific herring studied here both showed a decrease in swimbladder volume with increase in oil up to about 12% oil (Fig. 1) and this relationship was not destroyed by expressing both in terms of the volume of a gas- and oil-free fish. The range within which the swimbladder volume fluctuated was remarkably large: from 2.6% at 12.2% oil to 5.4% at 0.8% oil for fish 36 cm from the surface.

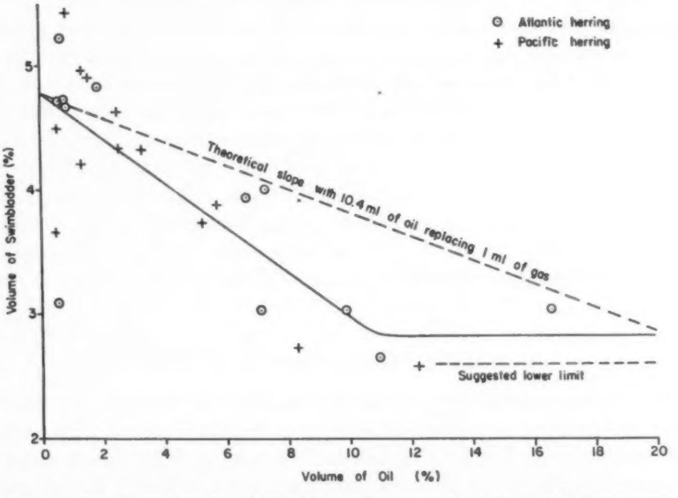

FIG. 1. Relationship of swimbladder volume to oil volume in two herring species. Volumes given as percentages of entire fish.

The replacement of gas by oil, however, was not perfect. It was calculated that 1 ml of gas provides the same upthrust as 10.7 ml of oil under the test conditions of the Pacific herring and as 10.4 ml of oil for the Atlantic fish. From lines fitted to the data for herring of 0 to 12% oil by the method of least squares it was calculated that 1 ml of gas was replaced by 5.1 ml of oil in the Pacific herring and by 6.1 ml of oil in the Atlantic herring over this range of oil contents. As the swimbladder is reduced in volume more than is justified by the increasing oil, and water is lost with increasing oil content as shown by Iles and Wood (1965), the fish becomes denser as the oil content increases up to about 12% oil.

Both herring groups appear to reach a lower limit to the decrease in swimbladder volume of approximately 2.6% at about 12% oil. Further increases in oil content up 16%, the highest value recorded, were not accompanied by further decrease in swimbladder volume. If this lower limit persists herring 36 cm from the surface would show a perfect replacement of gas by oil, and hence return to their density at no oil at about 23% oil volume or, allowing for changes in water content, at about 20% oil.

The effect of different oil contents and the accompanying change in swimbladder volume on the density of the fish is shown by the Atlantic herring, which when analysed fell into two groups (Table 1). The thin fish had been kept in captivity for 5 weeks before testing and had lost most of their oil whereas the fat fish were tested soon after capture. The fat fish had a lower mean swimbladder volume and had to be subjected to a greater reduction in pressure to attain neutral buoyancy. Four of the six fat fish tested liberated gas from the swimbladder before neutral buoyancy was achieved but at least two of these, and the two fish whose neutral buoyancy was determined, had densities and sinking factors above those of any of the thin fish. Calculations based on the changing swimbladder volume and water content of Atlantic herring with changes in oil suggest that density of the fish tested would decrease after attaining a maximum value at 12% oil. Since the density of the one herring with an oil content above this value was not obtained this feature is not shown in Table 1.

BUOYANCY OF HERRING IN NATURE

Wild herring usually show a diurnal pattern of vertical migration, being at or near the surface at night and at greater depths by day. The compression of swimbladder gas as the herring descend increases their density since herring cannot secrete gas into the swimbladder and seem unlikely to obtain it from stomach bacteria at temperatures present in the sea (Brawn, 1964). For Atlantic herring a line fitted by least squares to total fish density against oil volume gave a density of 1.0268 g/ml at 0% oil. The relationship of swimbladder volume to oil content for Atlantic herring of less than 12.8% oil is given by per cent swimbladder = 4.70 − per cent oil × 0.1644; hence the swimbladder volume at 0% oil would be 4.7%. The water content at 0% oil according to Iles and Wood (1965) is 79.4% by weight. Using these values the density of fish solids (whole fish less oil, water, gas) was found to be 1.536 g/ml.

To determine the density of herring of known oil content the swimbladder volume and water content were calculated from their known relationship to oil content assuming a swimbladder volume of 2.6% at all oil contents above 12.8% oil. Basing the calculation on 100-g herring the mass of solids was determined by subtracting water and oil from the total weight. The density of solids given above allowed the volume of solids and hence the volume and density of the whole fish at 36 cm depth to be calculated. The change in density and hence sinking factor with depth was then calculated for such a fish by allowing for the decrease in swimbladder volume with depth. Using such a method the sinking factors at various depths were calculated for herring of Passamaquoddy Bay, N.B. This area was the source of the Atlantic herring studied and the seasonal variation in many of its physical properties and the characteristics of its herring stock are known (Fig. 2).

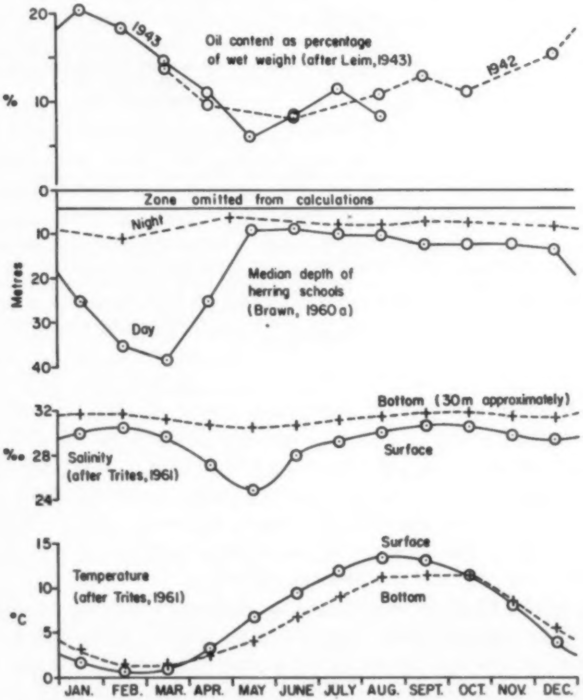

FIG. 2. Seasonal variations in properties of Passamaquoddy Bay, N.B., and of its herring stock.

In Passamaquoddy Bay in 1942–43 the Atlantic herring were fatter in February (18.4% oil by weight for herring without heads or tails, or 20.2% by volume) than in August (10.9% by weight, or 12.2% by volume) based on mean values (Leim, 1943; and Fig. 2). Using Leim's (1943) data for oil content and the corresponding water and swimbladder values the change in sinking factor with depth (Fig. 3) was calculated for these herring in February and August taking into account the seawater density calculated from the mean monthly salinities and temperature from Trites' (1961) data and the change in oil density with temperature. The main cause of the difference in sinking factor with depth between these two months (Fig. 3) is the difference in oil content. The increase in oil density at the low February temperature compared with that in August increases the sinking factor at the surface by about 2. A feature of the curves is that the herring in February, although much deeper in the water by day, has almost the same sinking factor (1022) at its median daytime depth as it has in August (1023).

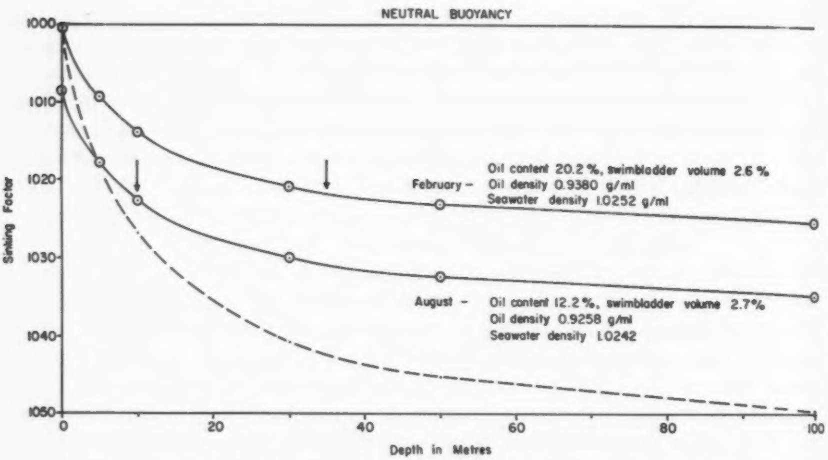

FIG. 3. Theoretical curves showing changes in sinking factor with depth in February and August for herring of Passamaquoddy Bay based on actual mean values of relevant variables. Arrows indicate the median depths to which the herring descend by day. They are at or near the surface at night. Broken line: sinking factors at increasing depths in February for a hypothetical fish with a 5% swimbladder volume and a sinking factor at the surface equal to that of a herring in February.

Herring of the Passamaquoddy region descend only to a moderate median depth of 25–38 m from January to April and to 9–13 m by day for the rest of the year (Brawn, 1960a). Other herring shoals go much deeper, an extreme depth of 500 m being reported by Devold (1963) for Atlanto-Scandian herring in arctic water in the East Icelandic current in autumn. These herring still returned to the surface at night. Assuming the arctic water had a density of 1.029 g/ml at 500 m and a temperature of 7 C it was calculated that the herring would have the following sinking factors at 500 m according to their oil contents:

Oil volume	10%	15%	20%
Sinking factor	1034	1027	1016

With 15% oil the sinking factor is comparable with that calculated for Passamaquoddy herring at the deepest part of their diurnal migration. It is known that experimental herring can compensate for such a sinking factor for at least a week. Oil contents of 15 and 20% are commonly found in herring and are well below the oil content of 29.8% recorded by Wood (1958) for a North Sea herring. Thus it seems when oil contents are high there is no need to postulate a mechanism for adding gas to the swimbladder below the surface to account for the reported depth range of the herring.

To demonstrate the importance of the low swimbladder volume of the herring in reducing the increase of sinking factor with increasing depth a curve is given in Fig. 3 for a hypothetical fish having a sinking factor of 1000.3 at the surface like the herring in February and the same seawater density but a swimbladder volume of 5%. This fish shows a sharp increase in sinking factor as it descends from the surface and it can only go down to 16 m for the same sinking factor that a herring in August shows at 100 m and that a herring in February would never attain by gas compression alone. Thus a herring has, by its reduction of swimbladder volume, attained a freedom of rapid downwards movement for a low increase in density which it could not have attained with a more typical swimbladder volume. In addition, since the herring does not secrete gas a return to the surface only restores the contained gas to its original surface volume. If some gas has been carried down from the surface in the stomach caecum and subsequently passed to the swimbladder through the pneumatic duct this excess gas can be rapidly expelled through the functional posterior duct of the swimbladder. Thus there also appears to be no restriction imposed by the swimbladder on the rate of upward movement.

This unusual freedom of vertical movement possessed by the herring could be of importance in escaping physoclist predators such as the cod, as suggested by Jones (1952). Based on his data for perch and wrasse he estimated that marine physoclists could move freely upward in a zone extending above their plane of equilibrium for a distance equivalent to a 22% reduction in pressure. Thus a cod adjusted to the pressure at 50 m could rise to 37 m; adjusted to 20 m it could rise to 13 m. As herring can swim vertically upwards without pausing to absorb gas and their maximum rate of swimming varies from 0.9 to 1.4 m/sec for lengths 15–27 cm (Brawn, 1960b), herring swimming upwards could theoretically move out of the range of cod or similar fish in 9–15 sec at 50 m and in 5–7 sec at 20 m.

The limit to rapid downward movement of physoclists, although not discussed in detail, is attributed by Jones (1951) to the resultant increase in density, which if excessive might carry the fish to the bottom. A consideration of the increases in sinking factor involved and the known ability of experimental fish to swim in midwater when all gas has been removed from the swimbladder (Copeland, 1952) casts doubt on this suggestion, especially during descents of short duration. For example, if a marine physoclist with a 5% swimbladder volume in neutral buoyancy with sea water of 1.027 g/ml had this gas compressed to the vanishing point, its density would become

1.081 g/ml and its sinking factor 1053, which although high is not much higher than the sinking factor of 1043 which mackerel apparently compensate for throughout their lives (Jones and Marshall, 1953). Even freshwater fish with the swimbladder gas completely removed can compensate for their increased density until secretion restores the normal volume (Copeland, 1952) although if we assume they had a 7% swimbladder volume this would require an initial compensation for a sinking factor of 1075.

The limit to downward movement of physoclist predators may be behavioural rather than physical. Such a behavioural limit would operate whenever negative stimuli such as increasing discomfort from the collapsing swimbladder or stimuli associated with increasing body density and perhaps decreases in light intensity outweigh the attraction to further downward movement. Such a limit would vary according to the physiological condition of the fish, nature of the downward attraction, past conditioning of the fish, and the present physical conditions of the environment.

Thus in discussing the modifications of herring in relation to downward escape from physoclist predators no fixed limits below which the herring would be safe can be given. However, at all depths below the surface the herring will have a greater sinking factor than a physoclist adjusted to that depth and the herring density might be increased further by the immediate release of gas through the posterior duct. This increased density could give the herring an initial advantage in the speed of downward movement.

The unusual features of the herring — its high oil content, low swimbladder volume, reduced skeleton, functional posterior swimbladder duct, and lack of a gas-secreting mechanism — combine to produce a fish with exceptional freedom of rapid vertical movement and the ability to remain at considerable depth below the surface for extended periods. In the herring the limitations of the physostome condition have been overcome not by the addition of a gas-secreting mechanism as in many physostomes but by the development of oil as an important buoyancy device. Thus the herring demonstrates another way in which the physostome condition can successfully be adapted to the demands of the environment.

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Seasonal Buoyancy Changes in Atlantic Salmon (*Salmo salar*) Parr and Smolt

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PINDER, LESLEY J., AND J. G. EALES. 1969. Seasonal buoyancy changes in Atlantic salmon (*Salmo salar*) parr and smolt. J. Fish. Res. Bd. Canada 26: 2093–2100.

A population of yearling Atlantic salmon parr was held in the laboratory from July to June in a negligible current at seasonal temperatures and photoperiods. Indirect measurements of swimbladder volume (pressure of neutral buoyancy) were made monthly on groups of 14–20 fish held for 24 hr either in still water or in a current (0.13 m/sec).

Parr were consistently less buoyant in the current than in still water. However, seasonal changes occurred, particularly in the current. For parr in a current, buoyancy was lowest in early winter but increased throughout the spring until the parr–smolt transformation, when smolts in the current were just as buoyant as smolts in still water. The seasonal development of smolt buoyancy in a current was related to body size and water temperature but did not seem to be affected by photoperiod.

The seasonal changes in swimbladder volume were not significantly correlated with tissue density (density of the gas-free fish), which in turn was not significantly correlated with the level of ether-extractable fat. However, the level of ether-extractable fat was significantly correlated with the coefficient of condition. At parr–smolt transformation, tissue density, level of ether-extractable fat, and coefficient of condition decreased sharply.

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INTRODUCTION

SAUNDERS (1965) showed that Atlantic salmon (*Salmo salar*) parr tend to lose swimbladder gases and tend to become more dense when water flow increases. Neave et al. (1966) substantiated this finding and also found that considerable swimbladder adjustment occurs within 12 hr after an increase in water flow. However, this response may not always be so evident nor without exceptions. Neave et al. (1966), for example, found that fry and parr lost less swimbladder gases in a current of water during December than during October. Moreover, Atlantic salmon smolts were found to be more buoyant than parr in a current and presumably did not have the same tendency to lose swimbladder gases (Saunders, 1965). Apparently, there is seasonal variation in the extent of swimbladder adjustment to a current of water.

An attempt has been made here to observe seasonal changes in buoyancy of Atlantic salmon in both still water and in a current for the 12-month period up to and including the parr–smolt transformation. Seasonal changes in the density of the gas-free fish and fat content were also measured, since these

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variables might influence the overall density of the fish and hence the swimbladder volume.

An attempt has also been made to determine the influence of spring seasonal changes in water temperature and daily photoperiod on the development of the buoyancy changes characteristic of smolts.

MATERIALS AND METHODS

Fish maintenance — Atlantic salmon parr were obtained in June 1965, November 1966, and November 1967 from the Miramichi Fish Culture Station, South Esk, New Brunswick. They were held in the laboratory in Fredericton city water and fed homogenized liver. Details of the temperature and photoperiod conditions used during each experiment are given in the Results and Discussion.

Measurement of pressure of neutral buoyancy on fish held in still water and in a current — The method and apparatus were similar to those used by Saunders (1965). Parr that had been confined in pairs for 24 hr in still shallow water in small containers were anesthetized with M.S. 222. One pair was placed in one of two chambers of a plexiglass flume through which a water flow was directed after the fish had revived from the anaesthetic. Another pair was similarly treated and placed in the second chamber which received a negligible water flow. Twenty-four hours later the flows were turned off and the fish killed with M.S. 222. Both chambers were sealed and simultaneously gradually decompressed until each fish had floated due to swimbladder expansion. That pressure at which the fish just floats is called the *pressure of neutral buoyancy*. For any given fish the pressure of neutral buoyancy is an index of swimbladder volume. The larger the swimbladder the more buoyant the fish will be and the greater will be the pressure of neutral buoyancy. Fish losing gases via the pneumatic duct during this procedure were discarded. The dead fish were weighed, measured (fork length), and the coefficient of condition (K) calculated according to the method of Hoar (1939), where K = mass (g) × 100/length^3 (centimeters). At the time of parr–smolt transformation the general extent of body silvering was visually assessed.

Determination of tissue density — From October onwards the gases were removed from the swimbladder of the dead fish with a hypodermic syringe and the density of the gas-free fish (tissue density) found by testing the fish in a series of saline baths of known densities (Harvey, MS, 1963). The series provided densities from 1.000 to 1.094 g/ml at 20 C in increments of 0.002 g/ml. The density of the fish was considered equal to the density of the bath in which it just floated. Since the saline baths were held at the same temperature as the holding tank and flume, whose temperatures varied seasonally, all density measurements were corrected for temperature. After the seasonal measurements, hydrometer determinations showed negligible alterations in the density of the saline baths from their initial level. Each fish was then frozen at –10 to –20 C.

Determination of per cent fat content of the fish — To determine the approximate fat content of the tissues, each frozen fish was thinly sliced and homogenized with three parts by weight of anhydrous sodium sulphate for each part by weight of fresh fish and 50 ml of petroleum ether (100 ml for fish over 10 g). Solids were removed by a medium porosity sintered glass filter containing activated charcoal and then washed three times with petroleum ether. The filtrate was evaporated to constant weight from tared glass petri dishes. The residue weight was expressed as a percentage of the fresh fish weight (% fat). This value would not represent all fats since petroleum ether does not dissolve all fatlike materials, particularly phospholipids.

RESULTS AND DISCUSSION

SEASONAL BUOYANCY CHANGES

A population of approximately 1000 yearling parr was held in the laboratory in a grey painted plywood tank (236 × 45 × 56 cm) from June 1965 until July 1966 at temperatures ranging from 7 C in midwinter to 14 C in June. In June 1965 the mean length of fish sampled was 6.0 cm (range 5.4–7.3

cm) and the mean weight was 1.7 g (range 0.9–3.0 g). By December the mean length had increased to 8.0 cm (range 6.3–10.0 cm) with a mean weight of 5.2 g (range 3.1–9.2 g), and by June 1966 the mean length was 11.7 cm (range 8.0–17.0 cm) and the mean weight 14.3 g (range 4.4–35.0 g). Incandescent illumination was regulated by a photocell corresponding to the natural photoperiod. Once each month, in a series of determinations lasting several days, the pressure of neutral buoyancy, tissue density, per cent fat, and K were measured for 14–20 fish held for 24 hr in still water and for 14–20 fish held for 24 hr in the current (Fig. 1).

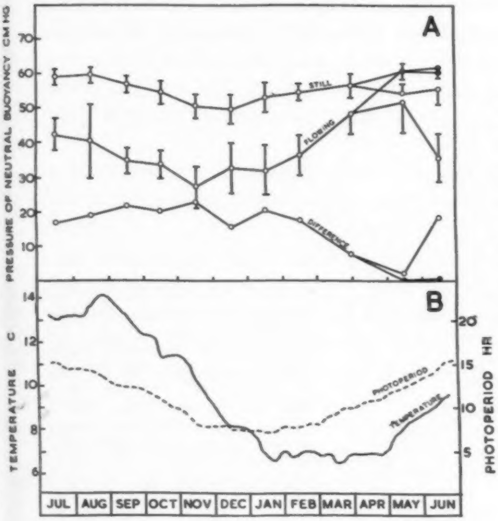

FIG. 1. (A) Seasonal changes in mean pressures of neutral buoyancy for parr and presmolts (○) (parr marks still visible) and smolts (●) (silvery with no parr marks visible) held for 24 hr in still water and for 24 hr in a current of 0.13 m/sec. Except in May and June when smolts were treated separately, each point represents a mean of determinations for 14–20 individuals. Two standard errors on either side of the mean are shown. The differences between buoyancy means for fish in a negligible flow and in the current are shown. (B) Seasonal changes in the daily photoperiod and water temperature.

For parr, pressure of neutral buoyancy measured in still water (PNB_s) was greatest in spring and summer and least in midwinter, suggesting that parr become denser in winter. However, the seasonal change in PNB_s could have been due to swimbladder compensation for seasonal changes in tissue density; the density of the parr with the swimbladder inflated need not have changed seasonally. This hypothesis could be tested most directly by measuring the density of the fish at different seasons with the swimbladder inflated. This was attempted but not pursued owing to the readiness with which swimbladder gases were lost during the density tests in the salt baths. However, the density of the gas-free fish was measured, and a very low negative correlation (r = −0.14, df = 86) between tissue density and PNB_s was obtained for individual parr between October and April. This suggests that seasonal changes in PNB_s cannot be explained by seasonal changes in tissue density and it is concluded that there are seasonal changes in buoyancy of parr in still water. This is probably related to changes in behaviour and adoption of a more bottom-dwelling mode of life (Lindroth, 1955; Saunders and Gee, 1964).

For parr, pressure of neutral buoyancy measured in a current (PNB_c) was also greatest in spring and summer and least in midwinter. Furthermore, the alteration in swimbladder volume due to exposure to a current ($PNB_s - PNB_c$) changed seasonally as well and was greatest in winter. Parr in a current seem to become denser in winter. This is presumably related to changes in behaviour and adoption of a more bottom-dwelling mode of life.

It had been anticipated that seasonal changes in tissue density might be inversely correlated with the per cent fat. The per cent fat rose from a mean of 1.4% in July to 3.4% in November and then declined to 1.2% for nonsmolts in June (Fig. 2). However, there was a very low negative correlation between the per cent fat and tissue density for parr between October and April ($r = -0.062$; df = 101). Clearly, either the ether-extractable fat has little bearing on the tissue density or the effect of fat is masked by changes in other factors. The coefficient of condition changed seasonally and there was a high correlation ($r = 0.74$, df = 216) between per cent fat and K values for individual parr from July to April, supporting the suggestion by Hoar (1939) that the coefficient of condition is a valid index of the fat content in Atlantic salmon.

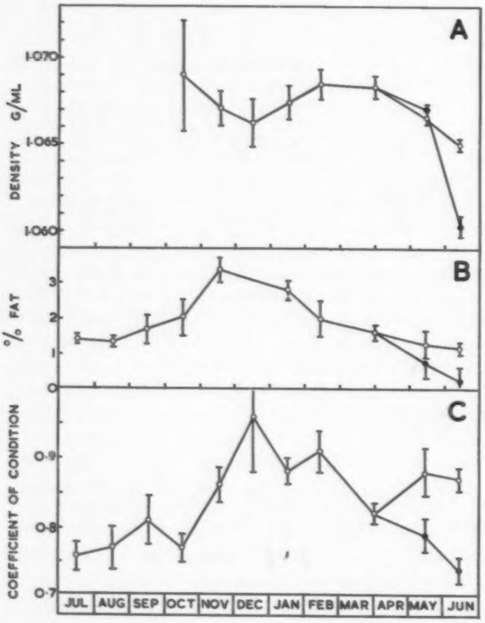

FIG. 2. Seasonal changes in tissue density (A), per cent fat (B), and coefficient of condition (C) for parr and presmolt (○) (parr marks still visible) and smolts (●) (silvery with no parr marks visible). Except in May and June when smolts are treated separately, each point represents a mean of determinations for 14–20 individuals. Two standard errors on either side of the mean are shown.

During parr–smolt transformation marked changes occurred for all parameters. Both PNB_s and PNB_c increased and the difference between them (PNB_s–PNB_c) was progressively reduced from February until May when almost identical readings for PNB_s and PNB_c were obtained. These data agree with the findings of Saunders (1965) that smolts in a current are more buoyant than parr and demonstrate, furthermore, that this difference is due at least in part to a negligible loss of swimbladder gases by smolts in response to an increase in water flow. Smolts may have been even more buoyant in both still and running water than recorded, since PNB values above 63.0 cm Hg were not measured and several smolts floated at that pressure.

Coefficient of condition, per cent fat, and tissue density decreased sharply at parr–smolt transformation. The decrease in per cent fat and K at this time agree with the findings of Lovern (1934) and Hoar (1939). The decrease in tissue density occurs despite a decrease in the level of ether-extractable fat and suggests changes in other aspects of tissue chemistry.

The Influence of Seasonally Changing Water Temperature and Daily Photoperiod on Buoyancy Changes at Parr–Smolt Transformation

In November 1966, 1000 2-year-old parr were randomly divided into four equal groups, each held in a grey rectangular plywood tank (236 × 45 × 56 cm). Ground liver (200 g) was fed per tank every 2nd day. The fish varied in size from a mean fork length of 12.5 cm (range 10.5–14.3 cm) and a mean weight of 18.0 g (range 10.6–26.4 g) in January to a mean fork length of 13.4 cm (range 11.0–15.5 cm) and a mean weight of 21.1 (range 12.4–31.8 g) by early April.

In late December the following photoperiod and temperature regimes were imposed on the four groups.

Temp (*C*)	Photoperiod	Code
3±1 from Dec. until Mar. 12 and then increasing to 14 by June	Lengthening from 8 hr in Dec. to 15 hr in June	NLNT
	8 hr Dec. to June	8LNT
3±1 from Dec. to June	Lengthening from 8 hr in Dec. to 15 hr in June	NL3T
	8 hr Dec. to June	8L3T

These temperature and photoperiod regimes are identical to those used by Johnston and Eales (1968) in their study of the environmental control of silvering. In fact the fish used in the present study were taken from the same experimental populations used by Johnston and Eales (1968), where greater detail of the holding conditions has been given.

At approximately monthly intervals, in a series of determinations lasting several days, the pressure of neutral buoyancy was recorded for 10–12 fish maintained for 24 hr in still water and for 10–12 fish maintained for 24 hr in a current of 0.13 m/sec (Fig. 3). The temperature and photoperiod conditions used in the flume were always identical to those in the holding tanks from which the fish had been removed.

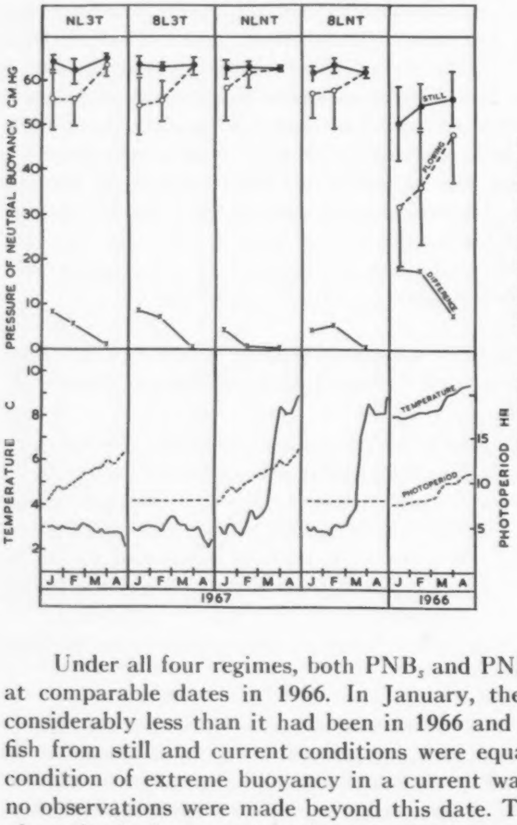

FIG. 3. Seasonal changes in mean pressures of neutral buoyancy for fish held for 24 hr in a negligible flow and for fish held for 24 hr in a current under four controlled temperature and photoperiod regimes. Each point represents a mean of determinations for 10–12 individuals. Two standard errors on either side of the mean are shown. The differences between buoyancy means for fish in a negligible flow and in the current are shown. A portion of Fig. 1B is repeated. The four environmental regimes of temperature and photoperiod from January 1967 to April 1967 inclusive are shown.

Under all four regimes, both PNB_s and PNB_c were greater than recorded at comparable dates in 1966. In January, the difference PNB_s–PNB_c was considerably less than it had been in 1966 and by late March or early April fish from still and current conditions were equally buoyant. Thus the smolt condition of extreme buoyancy in a current was reached by early April and no observations were made beyond this date. The buoyancy response typical of smolts had occurred at least 2 months earlier than in 1966.

There was little difference between the buoyancy of fish from the four temperature and photoperiod regimes, although fish from NLNT seemed more advanced. Inspection of the temperature records for the NLNT regime showed that these fish received slightly warmer water (within the range of 3 ± 1 C) than the other three regimes from December to March. This may have contributed the difference.

The advanced buoyancy response of the 1967 population could be due to the greater size of the fish used in 1967. It is also possible that smaller parr with a presumably less advanced physiological state might be influenced to some extent by seasonal changes in temperature and photoperiod. To investigate these possibilities further, the experiment was partially repeated between December 1967 and April 1968 using smaller fish, with fork lengths ranging from 7.5 to 10.8 cm in February. Four groups of parr were held under the same four photoperiod conditions used in 1966 and tested during February and March (before the temperature rise in NT regimes) and again during late March and April (after the temperature rise in NT regimes).

The results are shown below.

		Fish in a current				Fish in still water			
				Pressure of neutral buoyancy (*cm Hg*)				Pressure of neutral buoyancy (*cm Hg*)	
Date	Regimen	No. of fish	% silver	$\overline{X}$	SE	No. of fish	% silver	$\overline{X}$	SE
Feb. 10–16	NL3T	11	0.0	45.7	2.96	7	0.0	62.1	0.37
Feb. 24–26	8L3T	10	0.0	50.9	5.18	6	0.0	64.2	1.39
Feb. 28–Mar. 3	NLNT	9	0.0	49.9	5.40	6	0.0	62.4	3.12
Mar. 4–6	8LNT	10	0.0	51.5	4.87	6	0.0	63.1	0.91
Mar. 28–Apr. 2	NL3T	8	37.5	50.0	6.13	6	50.0	63.6	0.83
Apr. 5–6	8L3T	9	44.4	52.8	4.12	8	25.0	64.2	1.35
8–9	NLNT	8	100.0	63.6	0.01	6	100.0	63.6	0.01
8–10	8LNT	8	100.0	63.4	1.32	8	100.0	64.0	0.54

In these smaller fish the development of the increased buoyancy characteristic of smolts was less advanced, but there was evidence of environmental influence. The data clearly show that an increase in water temperature not only increases the incidence of silvering (as demonstrated by Johnston and Eales, 1968), but also hastens the development of the buoyant condition of smolts in a current.

The data support the conclusions of Johnston and Eales (1968) that photoperiod plays a negligible role in the regulation of certain aspects of parr–smolt transformation in Atlantic salmon.

It appears from this study and that of Johnston and Eales (1968) that body size and increase in water temperature are important variables influencing physiological changes at parr–smolt transformation in Atlantic salmon. These findings concerning the influence of size would support the observations of Allan (1944), Elson (1957), and Parry (1958, 1960) for Atlantic salmon, and those by Conte et al. (1966) and Vanstone and Markert (1968) on coho salmon, *Oncorhynchus kisutch*.

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Director, Biological Station, St. Andrews, kindly arranged the loan of an experimental flume. Dr P. F. Elson made critical comments on the thesis on which this paper is based. Mr P. B. Stratton, Fish Culture Station, South Esk, N.B., supplied the fish through the courtesy of Mr R. E. H. MacDonald, Fish Culture Branch, Maritimes Area. Dr J. H. Gee constructively criticised the manuscript.

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Changes in Serum Protein During the Molt and Reproductive Cycles of the American Lobster (*Homarus americanus*)

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Barlow, John, and George J. Ridgway. 1969. Changes in serum protein during the molt and reproductive cycles of the American lobster (*Homarus americanus*). J. Fish. Res. Bd. Canada 26: 2101–1209.

The variations in the serum proteins of lobsters were studied by quantitative protein determinations, starch gel and cellulose acetate electrophoresis, and immunodiffusion. A series of bleedings from 15 lobsters provided serum samples for each stage of the molt cycle. Total serum protein was highest before the molt and lowest directly after the molt. The total serum protein averaged 2.5 g/100 ml in the intermolt lobsters and variation among individuals was limited. Variation of serum proteins in an individual during the molt cycle included changes in the electrophoretic and immunodiffusion pattern. Quantitative changes during the cycle indicate a relative shift in the concentrations of various proteins. One component of serum protein was associated only with the development of eggs and another component was present only during premolt and directly after molt.

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INTRODUCTION

Serological and biochemical techniques have been used successfully to define reproductively isolated populations of many animals, including marine vertebrates (Cushing, 1963; Odense et al., 1966; Ridgway, 1962). These methods should be applicable to marine decapod crustaceans, if polymorphic characters can be found, and evidence can be obtained that the characters used are genetically controlled. We have been studying the serum proteins of the American lobster (*Homarus americanus*) in an attempt to separate phylogenetic variations from environmental and ontogenetic changes. The molt and reproductive cycles of Crustacea are accompanied by physiological changes including variations in hemolymph proteins (Damboviceanu, 1932; Drilhon, 1935; Glynn, 1968; Smith, 1911; Travis, 1955; Zuckerkandl, 1956). Although other tissues such as heart and muscle are less likely to reflect physiological variation, the use of serum proteins is desirable because of the ease of sampling and little harm done to the animal. The purpose of this paper is to describe changes observed in the serum protein of the lobster during the molt and reproductive cycles by electrophoretic, immunological, and chemical methods.

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MATERIALS AND METHODS

CLASSIFICATION OF THE MOLT CYCLE

Stages of the molt cycle were designated as described by Donahue (1954), but with descriptive modification by us to enable recognition of stages by external examination (Table 1). Stage A lasts from 6 to 36 hr, stage B from 3 to 6 weeks, stage C for 10 months or longer, stage D^1 from 1 to 3 weeks, stage D^2 about 1 week, and stage D^3 from 3 to 4 days. These time intervals depend on size of lobsters, sex, food, and environmental factors, such as temperature.

TABLE 1. Intermolt stages[a] of *Homarus americanus.*

Stage	Characteristics
A	Period of actual molt. New exoskeleton is completely soft and slippery to the touch.
B	Calcification. The exoskeleton assumes a papery consistency except the tubercles and spines, which are now hard. Throughout this stage, the exoskeleton becomes tough and leathery to the touch but remains compressible. The ventral surface of the abdomen changes from a dark color to white.
C	Completion of calcification and formation of thin membranous layer under the surface of the shell. The shell is hard to the touch throughout this stage. The ventral surface of the abdomen is white.
D^1	Preparation to molt. Abdomen just starts to change from normal white on ventral surface to pinkish. Carapace still hard along the ventral surface and proximal joints of the chelapids still hard.
D^2	Abdomen a dark red and carapace soft along the ventral lateral surface over the gill chamber due to absorption of lime salts.
D^3	Abdomen a dark purple, beginning posteriorly and becoming progressively darker as molt approaches. Proximal joints of the chelapids soften and ventral lateral surface of the carapace is soft. Close to molt.
	Stage D lasts 3–4 weeks. There is also softening along the middle line of the carapace leaving a narrow straight gutter extending from the rostrum to the posterior margin of the carapace.

[a] Stages described by Donahue (1954) modified by authors.

ANIMALS

Male and female lobsters taken near Boothbay Harbor, Maine, and ranging in carapace length from 56 to 94 mm were selected for their likelihood of molting (stage D^1 and D^2) and placed in running seawater tanks at the laboratory. Lobsters of the same size range were also selected in stage C and used as controls to test the effects of bleeding and handling. The animals were fed green crabs and herring. All animals were inspected weekly. Those in stage D^2 or D^3 were isolated and inspected daily.

BLEEDING AND COLLECTION OF SERUM

Lobsters were bled at least once in each stage of the molt cycle; time between bleedings ranged from 4 to 60 days. Bleedings of less than 4 days spacing were not attempted since Cole (1941) showed that more than two bleedings per week cause a significant decrease in the protein content of the hemolymph. We bled lobsters from the ventral abdominal sinus using a sterile 10-ml syringe with a 1.5-inch 22-gauge needle; 2–5 ml of hemolymph were removed and placed in a sterile airtight plastic cup to clot. The clot was broken with a wooden applicator stick, and the serum which was exuded in about 30 min was decanted into a plastic test tube and frozen for later analysis. No difference was noted between the frozen serum samples and serum exuded just prior to analyses. Notes on weight, carapace length, and stage of the molt cycle were recorded at each bleeding.

TOTAL PROTEIN

Total protein concentrations of the serum samples were determined by the method of Lowry et al. (1951). Lyophilized human serum and a single sample of lobster serum were used as standards in all determinations. Protein concentrations for some samples were adjusted to 3.2 g/100 ml for electrophoresis by pressure filtration at 5 C with 1 atm of pressure and then by dilution with distilled water.

IMMUNODIFFUSION

Antilobster sera were produced in 12 rabbits by immunization with male and female stage C lobster serum through subcutaneous, intraocular, and regional lymph node (with Freund's adjuvent) sites. All three immunization sites gave similar results.

A micro modification of Ouchterlony's (1958) method of immunodiffusion in agar was used (Ridgway et al., 1962). The diffusion medium consisted of Difco[1] Noble agar, 1.5; sodium chloride, 0.72; tri-sodium citrate dihydrate, 0.6; merthiolate Lilly, 0.01; trypan blue, 0.01; distilled water, 100. The *p*H of the medium was adjusted to 6.7 with hydrochloric acid. Citrate was used as the buffer to prevent precipitation of calcium salts. The slides containing the antigens and antisera were incubated at room temperature for 1–3 days after which the precipitation arcs were photographed by use of a dark field illuminator (Klontz et al., 1960). The slides were then washed to remove excess protein by covering with bibulous paper and saturating with 1% sodium chloride containing 1 cc of 10% thymol per liter as a preservative. The paper and wash solution were changed twice a day for 3 days. After a final washing with distilled water the slides were dried and stained with amido black and decolorized in a mixture of methanol, water, and acetic acid (5:5:1). These slides gave a permanent record with darkly stained precipitation arcs on a clear field.

ELECTROPHORESIS

Electrophoresis of serum was performed in starch gel by horizontal and vertical methods described by Smithies (1955, 1959), except that a Buchler gel form was used for the vertical electrophoresis. Hydrolyzed starch was used as prepared by Connaught Medical Research Laboratories, Toronto, Ont. A buffer (*p*H 8.6) was prepared by adding 20 g Tris-amino, 2 g disodium EDTA, and 3.8 g boric acid to sufficient water to give a final volume of 1 liter. Results were best when we used this buffer full strength for an electrode buffer and diluted to 0.3 original strength for a gel buffer. A variety of alterations of buffer composition and concentration yielded no better resolution.

After electrophoresis the gels were removed from their forms and sliced with a continuous-band stainless steel razor. The bottom half of the gel was stained for 5 min with amido black (2 g per liter) in a mixture of methanol, water, and acetic acid (5:5:1). The gel was then decolorized in several rinses of just 5:5:1. The top half of the gel was stained for peroxidase activity with 3.3′dimethoxybenzidine according to the method of Manwell and Baker (1963).

Electrophoresis was also carried out on cellulose acetate with a Beckman Microzone[2] system that uses the Beckman B-2 buffer and staining in Ponceau S. Several of the cellulose strips were also stained for peroxidase activity as described in the preceding paragraph.

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[1] Trade names referred to in this publication do not imply endorsement of commercial products.

[2] Beckman Instruments, Inc. Belmont, California

RESULTS

Quantitative Changes in Total Serum Protein

During the experiment 15 lobsters molted, and serum samples were obtained from all stages of the molt cycle. Protein determinations averaged about 6.5 g/100 ml protein before molting (stage D) and 1.7 g/100 ml at molting (stage A). At the end of calcification (stage B) protein concentration in the hemolymph built up to about 2.5 g/100 ml and remained at that level during the intermolt period (stage C). Variations of individuals from the mean level of serum were greatest before the molt and directly after the molt. These variations then decreased to a minimum and remained nearly constant throughout the intermolt (Fig. 1).

Six male and six female lobsters in stage C, averaging 500 g, were bled every 4 days for 40 days. Serum protein decreased after only one or two bleedings, but generally returned to normal and remained at about 2.5 g/100 ml for the rest of the bleedings.

Starch Gel Electrophoresis

Starch gel electrophoresis showed changes in protein concentrations and alterations in the electrophoretic pattern during the molt cycle. The starch gel electrophoretic pattern in the stage C lobsters consisted of eight bands (Fig. 2). Two of these bands exhibited peroxidase activity and are assumed to be hemocyanin although this was not confirmed by testing for copper content. Hemocyanin might also account for more than the two bands exhibiting the peroxidase activity by dissociation of subunits.

An alteration of the starch gel pattern, apparent after the molt, can be explained in part by dilution of the serum with water uptake at stage A. However, comparison of serum samples taken at various stages of the molt cycle, adjusted to a standard protein concentration of 3.2 g/100 ml, and separated by starch gel electrophoresis, revealed that certain components were affected more than others. Since the starch gel is not transparent and thus difficult to analyze quantitatively by densitometry, cellulose acetate electrophoresis was used to investigate further the effects of molting on the relative concentrations of the various serum proteins.

Cellulose Acetate Electrophoresis

The resolution of the cellulose acetate electrophoretic system was not as that in the starch gel and the bands are not directly comparable between the two methods. The cellulose acetate system does provide a very rapid method of analysis and is better able to be scanned by a densitometer. Although the affinity for the dye may vary between the various proteins the densitometer scannings do indicate changes in the relative concentrations of the proteins.

Serum protein patterns showed four principal bands on the cellulose acetate after electrophoresis (Fig. 3). Two bands were present in serums from all stages of the molt cycle. A third band was present only in stages D, A,

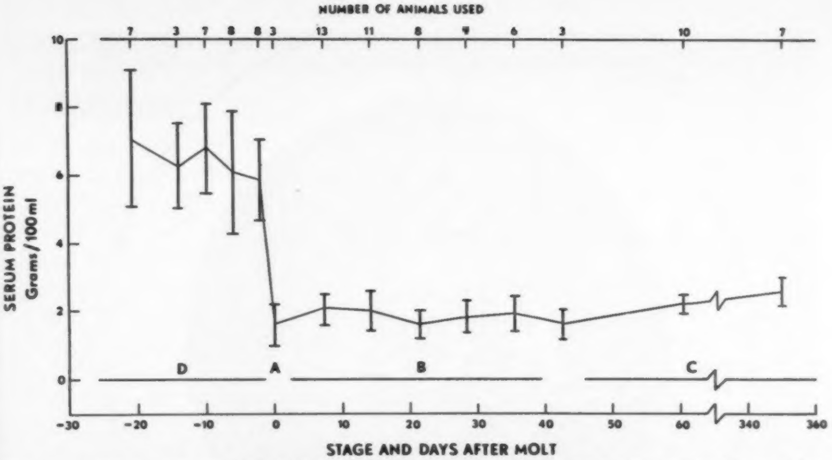

FIG. 1. Total serum protein during the molt cycle. The vertical lines indicate plus or minus the standard deviation of the mean.

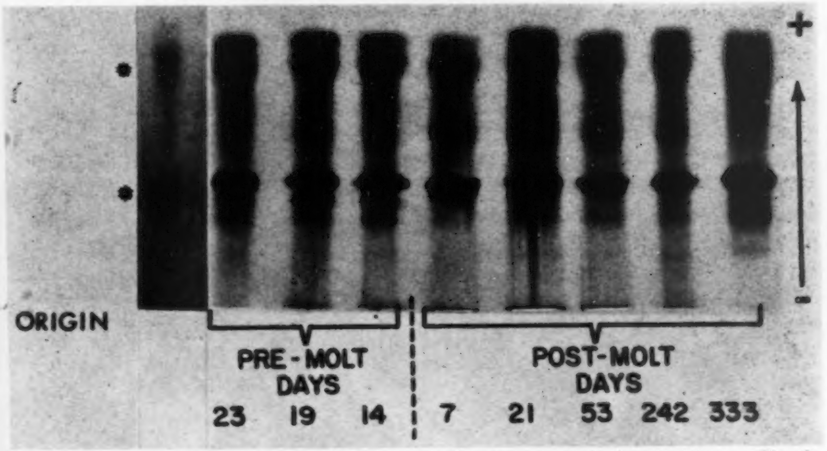

FIG. 2. Starch gel electrophoresis of serum samples from a single female lobster. The first sample is from a second gel stained for peroxidase activity (marked with asterisks (*)) (250 v for 4.5 hr, horizontal gel).

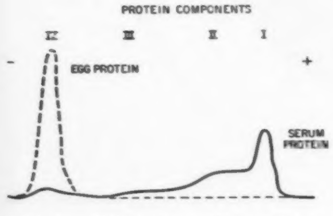

FIG. 3. Cellulose acetate electrophoresis (analytrol scan) of serum from early stage C female, and egg protein from the same female. The egg protein was run on the same cellulose acetate strip as the serum protein and scanned at the same speed (250 v for 20 min).

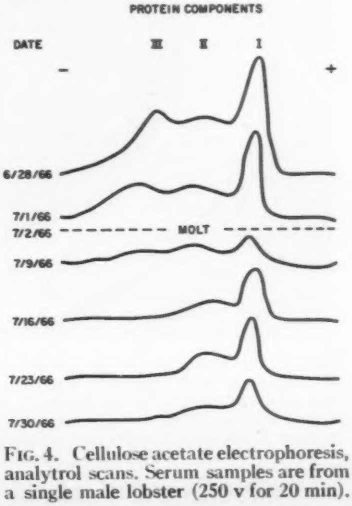

FIG. 4. Cellulose acetate electrophoresis, analytrol scans. Serum samples are from a single male lobster (250 v for 20 min).

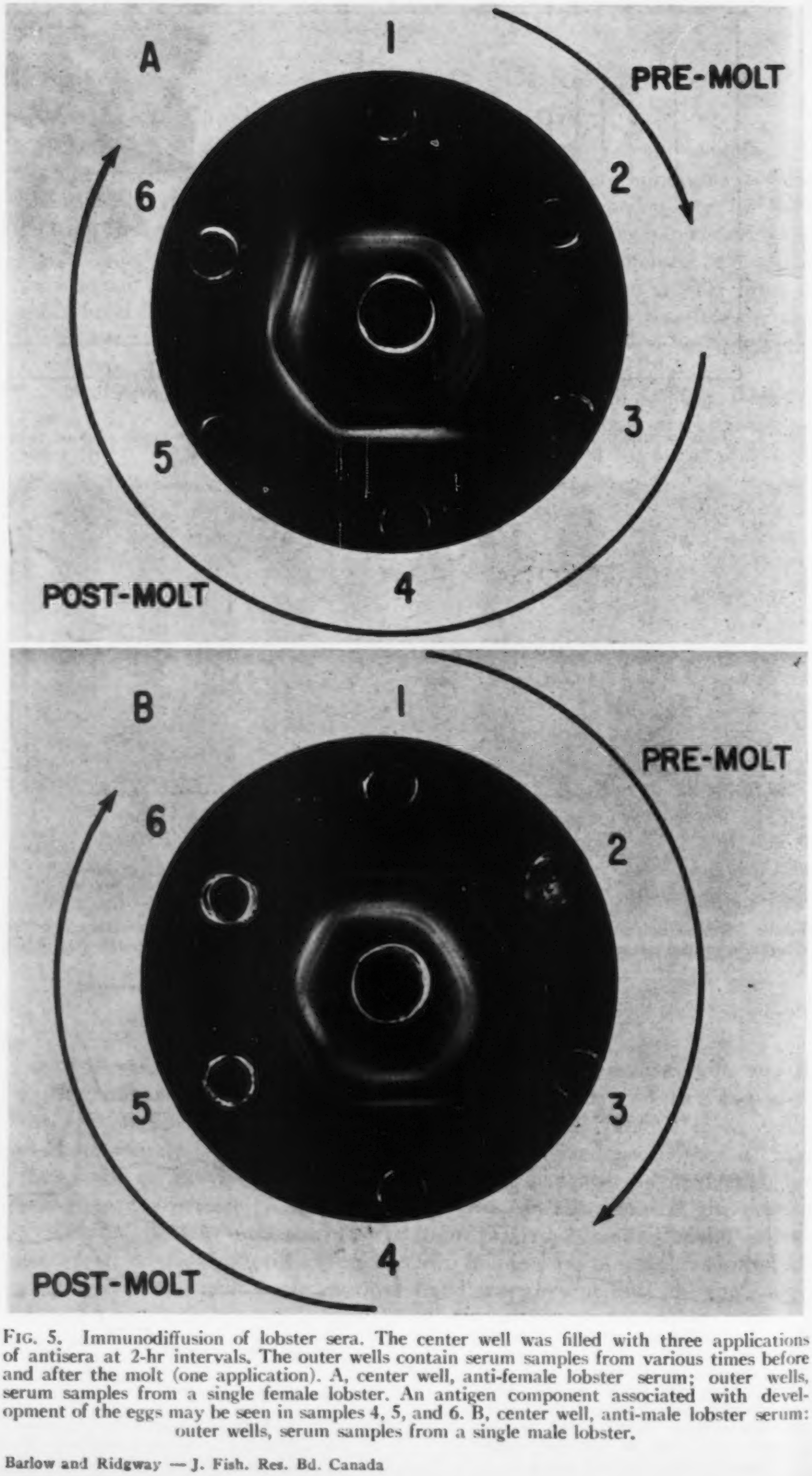

FIG. 5. Immunodiffusion of lobster sera. The center well was filled with three applications of antisera at 2-hr intervals. The outer wells contain serum samples from various times before and after the molt (one application). A, center well, anti-female lobster serum; outer wells, serum samples from a single female lobster. An antigen component associated with development of the eggs may be seen in samples 4, 5, and 6. B, center well, anti-male lobster serum: outer wells, serum samples from a single male lobster.

and B, and a fourth band only in females during the development of the eggs in the ovaries. This fourth band, present only in the female, was not observed in the starch gel system even with the same samples used to demonstrate the band on cellulose acetate; this may be due to a masking of the band by other protein components in the starch gel.

The analytrol scans indicated a relative shift in the concentrations of the various proteins during the cycle. Protein fractions were denoted I, II, III, and IV in order of decreasing mobility. Fractions I, II, and III were present throughout stages D and A. The concentration of these fractions increased before the molt. Fraction III disappeared during stage B, about 2 weeks after the molt. Fraction IV, associated with the eggs of the female, appeared about 1 month before the deposition of eggs on the swimmerets, and disappeared about a month later while the eggs still remained on the swimmerets. Comparison of egg homogenate and serum on the same cellulose acetate strip demonstrated that fraction IV had the same electrophoretic mobility as the principal egg protein. Changes in the relative concentrations of fractions I, II, and III are apparent from the patterns obtained from a single male lobster throughout a cycle (Fig. 4). Only fractions I and II remained detectable throughout the entire cycle, and peroxidase activity was also associated with these fractions.

The decrease in total serum protein in stage C lobsters was also detected by electrophoresis on cellulose acetate, but no shift in the relative concentrations of various components was noted. Components III and IV were not detected in the samples taken from control lobsters in stage C. This absence was to be expected since component III was only present during stages D, A, and part of stage B and component IV only in mature females during oögenesis. The electrophoretic pattern exhibited no alteration other than diminution of protein due to bleeding.

IMMUNODIFFUSION

We also studied the effect of the molting cycle on serum proteins using Ouchterlony's technique of double immunodiffusion. This technique permits simultaneous observation of numerous antigen-antibody reaction systems within the same reacting mixture. If several antigen sources (in this study different bleedings from individual lobsters taken during various stages of the molt cycle) are compared with a single antiserum, differences in the relative concentrations of any pair of antigens in the sources will be apparent from shifts in relative position of their precipitation arcs.

Utilizing the above method we observed a relative shift in the concentrations of certain antigens in the lobster sera with changes in the molt cycle. A minimum of six antigen antibody reaction systems were observed and changes were noted in both relative distances between the arcs and positions of the arcs with changes in the molt cycle (Fig. 5). The appearance and disappearance of several precipitation arcs during the cycle could be due either to a disappearance of that antigen from the serum or a dilution below the resolution of the technique. Both sexes possessed an antigen that disappeared about 1 month after the molt and appeared again in about 4 weeks. An additional system of antigens was present in the serum during development of eggs in the ovary; it was detected several months before egg deposition.

RELATION TO PUBLISHED RESEARCH

Quantitative Changes in Plasma Proteins during the Molt Cycle

Quantitative changes in plasma proteins during the molt cycle of crustaceans were first demonstrated by Drilhon (1935). Leone (1953) observed variation in total content of serum protein of lobsters, *H. americanus*, between 2.20 and 10.20 g/100 ml. Travis (1955) showed changes in total blood protein of *Panulirus argus* during the molt cycle that are similar to the changes we observed in *Homarus*. She reported an increase in blood protein preceding the molt in stage D, a decline after the molt in stage A, and subnormal values during the period of calcification, stage B. Glynn (1968) observed changes in the total serum protein concentration of *Homarus vulgaris* similar to those noted here in *H. americanus*, but in his animals the serum protein levels returned to high intermolt values within 24 days after the molt. The protein level in the serum of our lobsters failed to return to its premolt condition even after 350 days. This failure may have been due to a dietary deficiency or adverse temperature, for Stewart et al. (1967) have shown that hemolymph protein levels of *H. americanus* are intimately related to diet and temperature. Temperature probably had a pronounced effect in our lobsters since they were held in tanks supplied with local surface water which averaged 3.6 C for a 7-month period after the molt. At this temperature little growth and protein replacement could be expected.

Electrophoretic Studies on Crustacean Plasma Proteins

The plasma proteins of crustaceans have previously been investigated by a number of authors using electrophoretic methods. Stewart et al. (1966) reported that the free-boundary electrophoretic pattern of *Homarus* hemolymph has at least six components, one of which was identified as hemocyanin and one as fibrinogen. The three major components of lobster hemolymph were found to vary about equally with changes in plasma protein during starvation (Stewart et al., 1967). Woods et al. (1958) described the starch gel electrophoretic pattern of *Homarus*, using a sodium borate buffer solution. This system failed to resolve more than two components. Cowden and Coleman (1962) observed multiple copper-positive bands in a number of crustacean hemolymphs separated by starch gel electrophoresis and suggested that these bands represent distinct molecular types of hemocyanin. The two major protein bands that we observed showed peroxidase activity; it is likely that they represent two kinds of hemocyanin in the lobster.

Zuckerkandl (1956), who used paper electrophoresis, noted that the electrophoretic pattern of the crab (*Maia squinado*) varied during the molt cycle. Quantitative variation of electrophoretic components appeared as a relative shift in concentration before and directly after the molt. This change is similar to the variation we observed in the lobster. We have shown by direct dilution and concentration of the serum protein with pressure filtration that these variations among the protein components of lobster serums are not due solely to dilution or concentration. During the molt cycle, the components varied more or less independently to produce a characteristic pattern for the various stages. These relative shifts can be observed on starch gel electrophoresis but are more evident in the densitometer scannings from the cellulose acetate electrophoresis runs.

Serological Studies of Crustacean Plasma Proteins

Early serology of crustacean hemolymph dealt mainly with systematics of closely related species as tested by precipitation reactions (Leone, 1950). Denucé and Cushing (1963), working with the more sensitive immunoelectrophoretic procedure, were able to detect as many as nine components in the serum of *Panulirus interruptus*. These results also indicated that some crustaceans possess more than a single molecular species of hemocyanin.

The immunodiffusion method we used has provided further evidence for relative changes in the concentrations of protein components during the molt cycle. This method does not allow direct determination of protein concentration in the antigen components, but does allow a comparison of relative concentrations between serum samples; it has enabled us to show a definite shift in concentrations of serum antigens. Serial bleeding of control lobsters in stage C was followed by a decrease of total serum protein but this decrease was not accompanied by the relative shifts in protein concentrations that we found in molting animals.

Changes in Plasma Proteins Associated with Oögenesis

The production and storage of various proteins needed in oögenesis are also connected with changes in the protein patterns of the female lobster. The presence of a protein associated with egg formation in female crustaceans was first suggested by Smith (1911) in *Carcinus maenas*. This protein may be the same we noted in the lobster by cellulose acetate electrophoresis and immunodiffusion. Telfer (1954) discussed the occurrence of such a protein in the silkworm (*Cecropia*) and similar proteins have been widely demonstrated in fishes and birds (see Utter and Ridgway (1967) for pertinent references). The persistence of the protein in the hemolymph for over a month after deposition of eggs on the swimmerets may be explained by the absorption of materials from eggs that failed in passage; such absorption takes about 5 weeks (Herrick, 1911).

Physiological Basis of Variations in Plasma Proteins Associated with the Molt Cycle

The qualitative and quantitative changes in serum protein during the molt cycle reflect physiological modification throughout the body. The principal tissues concerned with molting are the hepatopancreas, blood, and integument (Passano, 1960). During premolt the absorption of portions of integument causes an increase in the concentration of blood constituents. Travis (1955) has suggested that amino acids available in hemolymph from absorption during premolt might also be utilized in resynthesis of new protein. The hepatopancreas acts as a storage organ for protein during the premolt period (Passano, 1960). During the molt, water uptake is necessary for increase in body size before calcification of the new exoskeleton begins. After the molt, organic reserves stored in the hepatopancreas and blood must be transported and deposited or used in the formation of the new exoskeleton. We found serum protein values during deposition subnormal and variable. Utilization and transport of the individual proteins involved in molting may differ among proteins and cause the shift in relative concentrations we observed in the lobster.

SIGNIFICANCE OF FINDINGS

The total serum protein of the lobster builds up before the molt, is diluted at the molt, fluctuates during calcification, and remains nearly constant during the intermolt period. We have shown, using electrophoretic and immunological techniques, that there are relative shifts in the individual serum protein components. One protein component varied with the reproductive cycle of the female, and others varied in relative concentration with the molt cycle. One component separated by electrophoresis on cellulose acetate was present only before and shortly after the molt, and one antigen system disappeared about a month after the molt but returned in about 4 weeks.

Differences in plasma proteins among lobsters might be attributed to either physiological changes associated with the molt or reproductive cycles, or to genetic polymorphism. The significance of our findings is that variations have been found which are associated with the molt cycle and, in the female, with sexual maturation. These variations must be taken into account before differences among lobsters are attributed to genetic causes.

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Olfactory Discrimination of Natural Waters by Salmon

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Three sets of experiments are described, all based on the electrical responses recorded in the olfactory bulb of salmon (*Oncorhynchus tshawytscha* and *O. kisutch*) when various natural waters, or modified natural waters, were infused into the nostril.

In the first experiments it was found that, generally, the most vigorous response was evoked by the home spawning area water and relatively less vigorous responses were evoked by waters in which the tested salmon had never been. However, an exceptional situation was found in which a strange water (Issaquah River) evoked olfactory responses in Soos Creek and University of Washington salmon that could not be distinguished from the home water response.

Chinook salmon that had been reared in a freshwater hatchery for 2 years and then placed in sea water for 2 weeks retained their home water response in sea water. Placing chinook salmon in a small volume of fresh water, which had previously evoked only a slight bulbar electroencephalographic (EEG) response, rendered the water highly stimulatory. Keeping coho salmon under the same conditions did not make the water as stimulatory.

Two-year-old adult chinook salmon placed in a new environmental water that was relatively weakly stimulatory at first developed a bulbar EEG response to the novel water in 3 days that was not distinguishable from the home water response.

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INTRODUCTION

OLFACTION, and presumably olfactory memory, in salmon has been shown to be indispensible for successful homing (Wisby and Hasler, 1954; Sato et al., 1966). Further analysis of olfactory function in salmon by Hara et al. (1965) and Ueda et al. (1967) using electroencephalography provided the information that nasal infusion of "home water" provokes a characteristic electrical response in the olfactory bulb. Ueda et al. (1967) showed that although waters traversed by salmon during their migration are stimulatory to the olfactory bulb, they are inferior in this respect to home water. Completely strange waters, in experiments by Ueda et al., were not stimulatory, opening the possibility that the bulbar response is highly discriminatory and is therefore a valuable tool in further analysis of olfactory guidance of anadromous salmon.

In continuing the work of Ueda et al. we have tried to improve experimental conditions by eliminating the transport of the salmon from the field to the

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laboratory and thus avoid temperature changes and some stress factors. Furthermore, in some preliminary work (unpublished) we found that the ability of a water sample to evoke an olfactory bulbar response diminishes gradually at room temperature. For this reason we felt that additional experiments should be done with natural water samples collected on the day of use and refrigerated until used.

Results of these tests, under different conditions, vary from those reported by Ueda et al. (1967) in certain important respects. Furthermore, some of the olfactory tests were made on chinook salmon raised for 2 years in a freshwater hatchery before being placed into sea water for several weeks. Although data from such salmon have no normal counterpart, they may provide some insight into the properties and character of the evoked electrical olfactory bulbar response of normal salmon.

MATERIALS AND METHODS

Spawning, adult salmon weighing 3–15 kg were taken at three different spawning grounds: 13 chinook salmon (*Oncorhynchus tshawytscha*) from University of Washington Fisheries pond in Seattle, Wash.; 12 coho salmon (*Oncorhynchus kisutch*) from Green River State Hatchery near Auburn, Wash.; and 8 coho salmon from the Issaquah State Hatchery near Issaquah, Wash. Testing equipment in each instance was set up in a building immediately adjacent to the source of animals to be tested. Temperatures were close to those prevailing out of doors at each place.

A number of 2-year-old chinook salmon, which were reared in a small hatchery in flowing well water adjacent to the Willamette River at Corvallis, Oregon, were used in another group of tests. Three of these were tested with no further treatment; four were tested after an additional 2 weeks in recirculated artificial sea water in the laboratory of Dr F. P. Conte, Oregon State University, Corvallis, Oreg.

In an experiment designed to test for adaptation to a new water in the development of the electrical olfactory bulbar response, 16 adult chinook salmon were taken from the spawning area in Soos Creek adjacent to the Green River State Hatchery and transported to the University of Washington College of Fisheries. Here they were kept in pairs in 100-gal glass aquaria in flowing aerated well water plus water from Portage Bay, the adjacent body of natural water. They were tested in an unheated building about 75 yards distant.

Immediately after removal from the pond or the aquaria, fish were anesthetized lightly by immersion in tricaine methanesulphonate (M.S. 222, 1%) and immobilized with an intramuscular injection of 2.0 mg/kg gallamine triethiodide. The fish were placed in a fitted plastic trough and the gills were perfused with tap or spring water (5.0 liters/min, at 8–9 C). The olfactory bulb was exposed by rapid dorsal dissection and a stainless steel bipolar electrode, prepared as previously described (Oshima and Gorbman, 1966), was placed on the left posterior surface of the olfactory bulb with the aid of a micromanipulator and a dissecting microscope. The various natural water samples were infused intranasally at the rate of 1 ml/sec. Infusion duration was 15 sec followed by a 15-sec rinse with distilled water. All tested waters were obtained fresh daily and held at 6 C. In some fish, the olfactory tracts were transected bilaterally.

Electroencephalographic patterns (EEG) were recorded with a Model D Grass polygraph equipped with a 5P5C preamplifier. Quantification of electrical activity of the responses evoked by nasal stimulations was obtained simultaneously by an electronic integrating circuit (Grass 5P3 integrater). This instrument sums up the total deviation from the baseline of the EEG trail and records the function as a single line. By measuring the area under the integrated curve a representative quantitative value could be obtained. Measurements of the integrated area during the first 10 sec of a 15-sec olfactory stimulation were made by the same technician using a K & E compensating Polar Planimeter.

RESULTS

SPECIFICITY OF THE RESPONSE TO HOME WATER IN ADULT SPAWNING SALMON

Figure 1 represents the olfactory bulbar electrical responses to three natural waters of an adult spawning chinook salmon which had homed into the

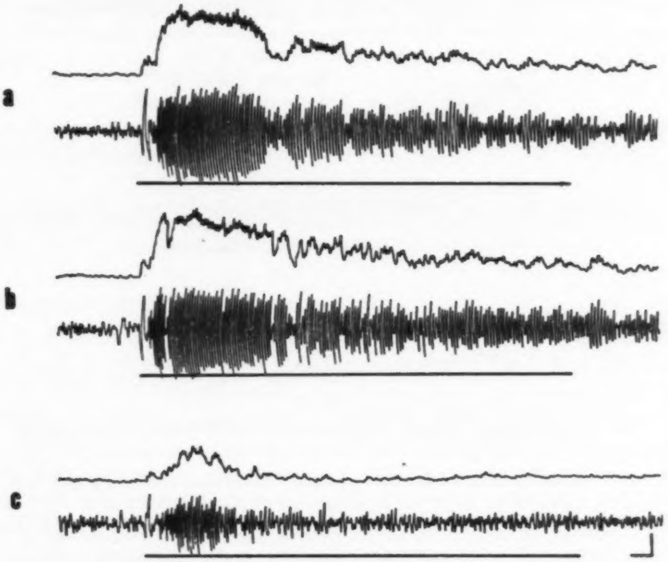

FIG. 1. Electrical olfactory bulbar response to various water samples by University of Washington Fisheries chinook salmon (October 1966). Upper trace is integrated EEG; lower trace, EEG. *a*, Response to home water; b, response to Issaquah Creek water; *c*, response to water from Soos Creek. Duration of stimulus is indicated by the heavy line under lower trace. Calibration (at lower right): horizontal, 1 sec; vertical 50 μv.

Fisheries pond at the University of Washington campus. The synchronized high amplitude response to home water can be distinguished easily from Soos Creek water, but not from Issaquah Creek water. The bulbar responses in Soos Creek coho salmon evoked by Skykomish water are of lower magnitude than the home water response. On the other hand, the bulbar responses to Issaquah water cannot be distinguished from the home water response. Hence, the bulbar responses to Issaquah water in both University of Washington chinook and Soos Creek coho are indistinguishable from respective home water responses.

Individual responses and statistical analyses of integrated bulbar EEG responses to various natural waters in adult spawning salmon are summarized in Table 1. These averaged data show that except for the strong response of Soos Creek coho salmon and University of Washington chinook to water from Issaquah, home water responses are strongest. However, even the feeblest

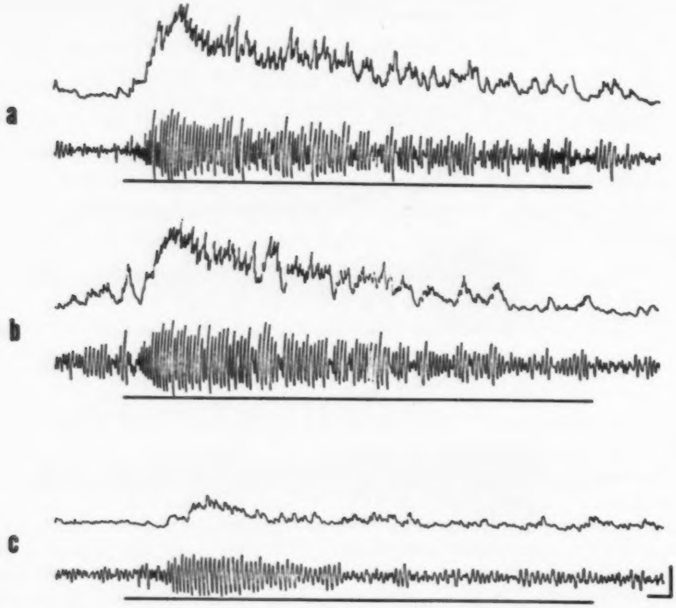

FIG. 2. Electrical olfactory bulbar response to various water samples by Soos Creek coho salmon (December 1966). Upper trace is integrated EEG; lower trace, EEG. *a*, Response to home water; *b*, response to water from Issaquah Creek; *c*, response to water from Skykomish Creek. Duration of stimulus is indicated by the heavy line under lower trace. Calibration: horizontal, 1 sec; vertical, 50 μv.

averaged response to a strange (nonhome) water was almost 50% of the home water response.

EEG Olfactory Discrimination in Hatchery-Reared Chinook Salmon at Corvallis, Oreg.

These salmon had been kept for 2 years at the Willamette River State Hatchery in water from a flowing well about 75 yards from the Willamette River. About 2 weeks before these tests, some of these animals were brought to the Radiation Biology Building, Oregon State University, and placed in plastic tanks containing either recirculating filtered artificial sea water or dechlorinated tap water derived from local mountain drainage. The temperature of the tank waters was regulated at 9–10 C.

Regardless of whether they were kept in salt or fresh water, these salmon retained the ability to react strongly to "home water" (Fig. 3) that, in this instance, was water taken directly from the rearing tank. Relative to the home water response, all tested fish reacted very weakly to the headwaters of a small local stream, Oak Creek (Table 2). However, if two salmon were placed in

TABLE 1. Specificity of the response to home water in adult spawning salmon.

	Relative response[a]		
Test water	Univ. Wash. Fisheries chinook	Soos Cr. coho	Issaquah Cr. coho
Home water	100.0 (N=7)	100.0 (N=7)	100.0 (N=8)
Soos Cr.	48.8±8.5 (N=13)	–	63.1±10.4 (N=4)
Issaquah R.	101.6±9.3 (N=12)	99.0±10.5 (N=10)	–
Skykomish Cr.	66.3±8.1 (N=8)	73.1±6.3 (N=12)	–
Univ. Wash. Fisheries pond	–	–	74.5±6.5 (N=6)

[a] Values are the mean ratios (×100) of the magnitudes of the home water response to the magnitudes of the nonhome water responses. The magnitudes, as explained in the Methods section, are derived from measurements of electronically integrated EEG curves.

about 5 gal of Oak Creek water overnight, the olfactory stimulation by this water was increased greatly. It is interesting that these young chinook salmon reacted much more strongly to Oak Creek water if two chinook salmon had remained in it than if two coho salmon had been used for this purpose. However, it is interesting also that they reacted more strongly to the artificial sea water if they had lived in it for 2 weeks than if they had not. This recirculated sea water, though its volume relation to the fish was much larger, also had been "lived in" by a number of chinook salmon in several tanks. In summary, it was clear that these 2-year-old migratory chinook salmon can retain their "home water" responses at least 2 weeks and that they can readily distinguish home from other fresh water. Furthermore, prior residence of a salmon of the same species in a water greatly increases the olfactory bulbar stimulation produced by a particular water. Beyond this, it should be noted that saltwater-habituated chinook salmon reacted more strongly to their new environmental sea water than did chinook salmon that had never been in such recirculated sea water.

Adaptability of Olfactory Bulbar Responses of Chinook Salmon to a New Environmental Water

Electrical bulbar responses to Soos Creek supply water and to University Fisheries pond supply water were tested 0, 1, 3, 5, and 13 days after transport from the Green River State Hatchery pond (Soos Creek) to aquaria at the University of Washington College of Fisheries. In these aquaria there was a flow of aerated water from the adjacent Portage Bay, on the canal between Lake Washington and Puget Sound.

Representative bulbar EEG responses to both home water and the new environmental water are illustrated in Fig. 4. Table 3 summarizes the results of these "adaptation" experiments. It can be concluded from these data that the olfactory responses increase from an initial low value until about 3 days

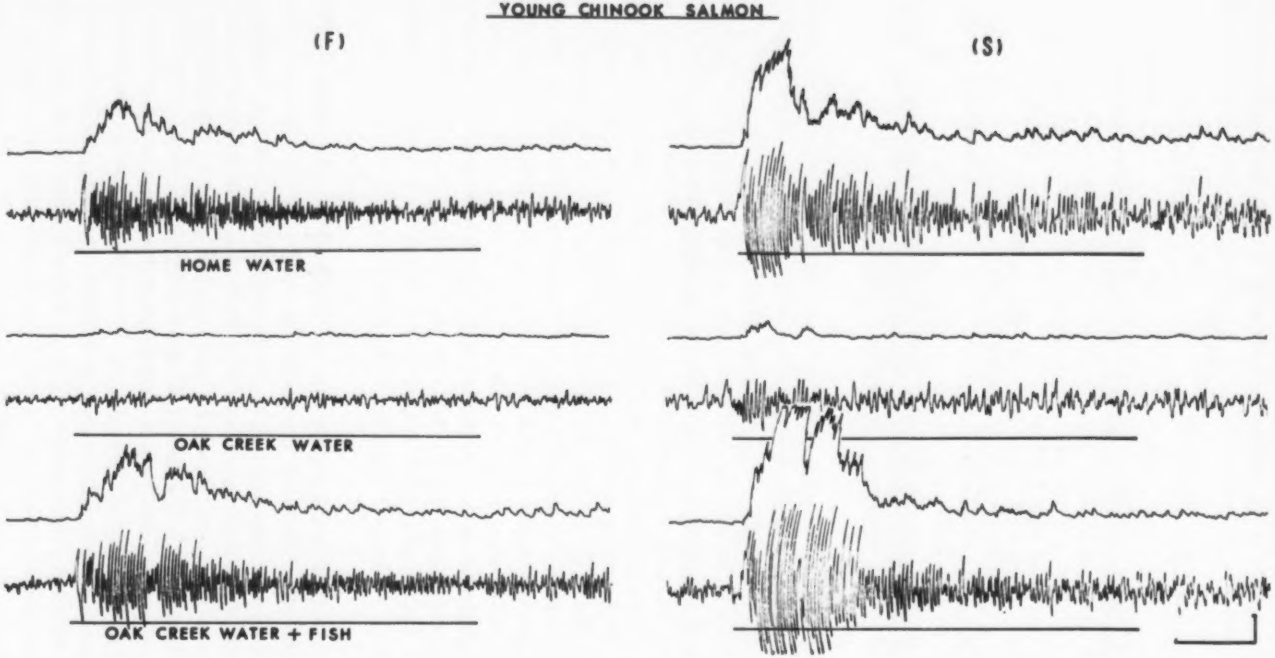

FIG. 3. Olfactory bulbar EEG responses to various water samples in 2-year-old chinook salmon at Corvallis, Oregon. Experiments were carried out in the Department of Radiation Biology, Oregon State University, Corvallis, June 18–21, 1967. The fish were taken from a tank filled with well water from a point adjacent to the Willamette River (F) or from an artificial seawater tank in which they had stayed 2 weeks (S). Water temperatures were controlled at 9–10°C. The line under lower trace (EEG) indicates stimulus duration. Upper trace is integrated EEG. Oak Creek water + fish refers to Oak Creek in which two chinook salmon had been kept for 24 hr. Calibration: horizontal 5 sec; vertical 50 μv.

TABLE 2. EEG olfactory discrimination in hatchery-reared 2-year-old chinook salmon at Corvallis, Oregon. This experiment was carried out from June 18 to 21, 1968.

		Relative response[a]		
	Home water	Oak Cr. water	Oak Cr. water + fish	Salt water
Kept in salt water	100.0 (N=4)	23.2±4.7 (N=4)	135.0±21.0 (chinook N=2) 60.0±12.4 (coho N=2)	117.0±3.5 (N=2)
Kept in fresh water	100.0 (N=3)	28.0±7.5 (N=3)	166.0±14.4 (N=2)	75.0±12.6 (N=3) $P < 0.05$

[a] Values are the mean ratios (×100) of the magnitudes of the home water response to the magnitudes of the nonhome water responses.

after transfer, when the response to the new water cannot be distinguished from the home water response.

This experiment was carried out in December 1967. Similar, but less complete observations were made in 1966 when we tested adult spawning coho salmon transferred from Soos Creek to the University of Washington.

EFFECT OF BILATERAL OLFACTORY TRACT SECTION ON BULBAR EEG RESPONSE IN ADULT SPAWNING SALMON

After bilateral olfactory tract section, the integrated value of bulbar responses to home water decreased to 15–25% of the values before section, and a depressive effect from distilled water washing persisted. Thirteen chinook salmon which had migrated into the University of Washington College of Fisheries pond were tested for their olfactory bulbar EEG response to Soos Creek water and to University of Washington Fisheries pond water, both before and after olfactory tract section. Tract section isolates the olfactory bulb from the rest of the brain but still permits afferents from the nasal mucosa to reach the bulb.

The patterns of the responses were altered, but the mean ratio before section (48.8 ± 8.5%) was not significantly different from the ratio of response of home to Soos Creek water after section (42.3 ± 9.2%). These results indicate that although there is some centrifugal influence of more posterior parts of the brain on the salmon olfactory bulb, even when it is isolated from secondary (more posterior) olfactory centers, discriminative responses still can occur in the olfactory epithelium–olfactory bulb part of the system.

DISCUSSION

The basic conclusion of Hara et al. (1965) and Ueda et al (1967) was that relatively specific electric responses can be recorded from the olfactory bulb

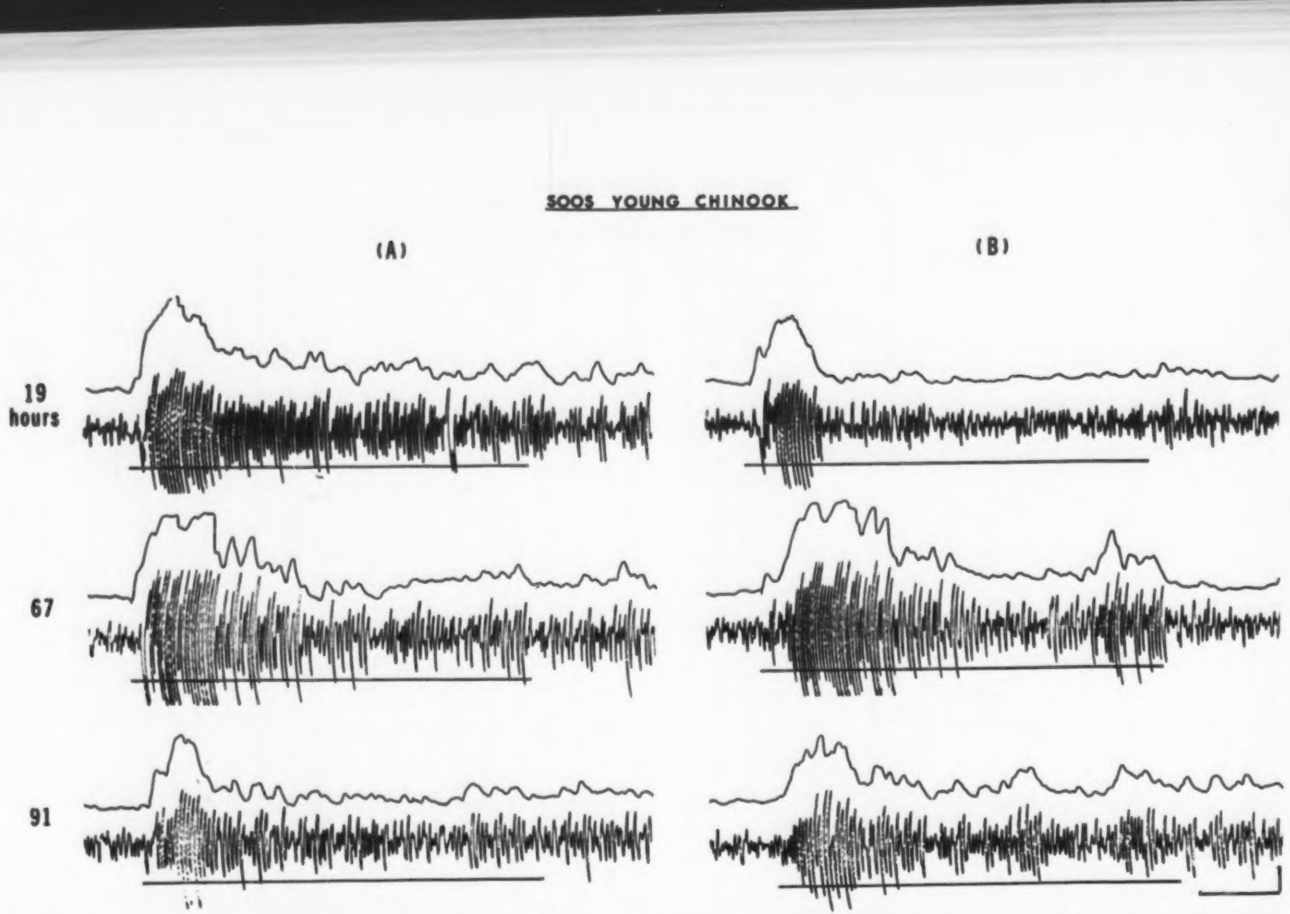

FIG. 4. Effect of environmental change in which 2-year-old chinook salmon from Soos Creek Hatchery, Auburn, Washington, were tested for olfactory bulbar EEG responses after variable times in aquaria at the University of Washington College of Fisheries in local water. Numbers at left indicate the total time (hours) after introduction into the new water. *a*, Responses to Soos Creek water; *b*, Responses to University of Washington College of Fisheries pond supply water. Upper trace is integrated EEG; lower trace, EEG. The lines under the lower trace indicate the duration of stimulus. Calibration (shown at lower right): horizontal 5 sec; vertical, 50 μv.

TABLE 3. Adaptability of olfactory bulbar responses of 2-year-old chinook salmon to a new environmental water.

Time after transfer (*hr*)	No. of fish	Bulbar EEG response ratio $\frac{\text{Univ. Wash. Fisheries}}{\text{Soos. Cr.}} \times 100$
0	3	44.1±1.1(A)[a]
17–22	5	62.0±6.3(B)
67–70	3	123.6±10.4(C)
91–93	2	117.8±1.6
13 days	3	107.8±9.8(D)

[a] (A) vs. (B) not significant; (B) vs. (C) $P < 0.025$; (C) vs. (D) not significant.

of salmon when certain natural waters are infused into the nose. They found the responsiveness of the olfactory bulbar electrical activity was virtually limited to water from the natural spawning area of any particular salmon, but that some responsiveness could also be found to waters traversed by the fish in ascending to the spawning area.

In the broadest sense, our experiments utilizing less debilitated fish, and test water samples as fresh as possible, confirm those of Ueda et al. (1967). However, there are important differences between the results described here and the earlier ones of Ueda et al. that should be discussed more fully. Although we found that the electrical olfactory bulbar response is discriminatory, it does not necessarily discriminate home water from waters which the salmon has not experienced before. For example, bulbar responses in Soos Creek coho and University of Washington chinook evoked by Issaquah River water and respective home waters were of the same magnitude. The Issaquah water response was the only real exception, however, to the generalization that the home water response is strongest.

Another difference between our results and those of Ueda et al. (1967) was in the degree of discrimination. The ratio of the home water response to the responses to other natural waters was generally less than 0.1 in Ueda et al.'s tests. In our tests (except for the instance already mentioned) it generally was 0.5 to 0.75. We can only ascribe these differences to the relative physiological conditions of the test animals and to the relative freshness of the tested water samples. Ueda et al. transported the salmon, which were usually in poor condition to begin with, in a stainless steel tank over 40 miles, and retained them in a nonhome water environment in glass aquaria sometimes over a period of several days and at temperatures usually in excess of their normal temperature. Mortality under these conditions was appreciable, although it was not actually measured by Ueda et al. It is quite possible under these conditions, when all olfactory responses are relatively lower in amplitude, that the relatively weaker responses (to nonhome waters) are virtually lost. This would explain the low ratios mentioned above.

The experiments with 2-year-old hatchery-reared salmon are instructive because they show that residence in artificial sea water for 2 weeks does not appreciably affect the home water response, or the ratio of discrimination of home water against another fresh water. This is of interest because we have never before tested a home-water imprinted fish while it was in sea water. Obviously, we cannot carry very far the comparison between the laboratory test conditions at Corvallis and the natural freshwater–saltwater sequence in the normal life cycle. However, it is extremely unlikely that we shall ever be able to solve the practical problems to test the olfactory responses of salmon to their home waters from their natural salt water environment. At the very least then, this experiment demonstrates retention of the home water electrical response in the olfactory bulb through a period of residence in sea water.

Evidence for olfactory species-specificity can be taken from the data in Table 2. Here it is seen that water (Oak Creek) that itself has a very low olfactory stimulatory value can be made highly stimulatory for the chinook olfactory bulb by keeping chinook salmon in it overnight, but less so if coho salmon are kept in it in the same way. This experiment, though inconclusive, is reminiscent of the report by Todd et al. (1967). They found that catfish can recognize odors of other individual catfish, and respond appropriately when small amounts of such water are placed in the aquarium. This experiment also suggests that the olfactory elements in the "home water" of the Corvallis chinook we tested are largely derived from the fish themselves since freshly drawn well water, which continuously supplied the rearing tank, evoked only weak responses similar to those evoked by Oak Creek water.

The experiments on adaptation of the olfactory response to a new environmental water (summarized in Table 3) indicate that even spawning adults can develop an altered or even new electrical response to a novel environment. For this experiment we selected a test combination which gave the lowest starting ratio of integrated responsiveness: $\frac{\text{novel water response}}{\text{home water response}}$. Over a period of 3 days this ratio increased from an initial value of 0.4 to equal or slightly exceed the home water response.

ACKNOWLEDGMENTS

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Electroencephalographic Olfactory Responses in Adult Salmon to Waters Traversed in the Homing Migration

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OSHIMA, KIYOSHI, WILLIAM E. HAHN, AND AUBREY GORBMAN. 1969. Electroencephalographic olfactory responses in adult salmon to waters traversed in the homing migration. J. Fish. Res. Bd. Canada 26: 2123–2133.

By infusing various natural waters into noses of homing salmon (*Oncorhynchus tshawytscha* and *O. kisutch*) and recording electroencephalographic (EEG) patterns from the olfactory bulb, data were collected showing responsiveness to waters along the freshwater migratory route. The largest responses always were evoked by the home water for each of the three spawning groups. Generally, the responses to various waters along the migrating route were equivalent, or did not vary in any recognizable progression. Accordingly, it does not seem that homing salmon are following a single olfactory clue in increasing concentration. Instead, they appear to be responding olfactorily to individual loci along the route.

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INTRODUCTION

INCREASINGLY COMPLETE DESCRIPTIONS of the remarkably precise migrations of salmon have not been matched with progress in the analysis of what orients these animals during their migrations. The only experimental approach to this question has been simple blockage of the noses as employed by Wisby and Hasler (1954) to show that without normal olfactory function, successful anadromous migration is impaired. Groot (1965) has used test situations to find that the catadromous migration of juvenile sockeye salmon is guided by a complex of factors in which celestial clues and polarized sunlight are important guides.

Recently, another experimental tool has become available from the work of Hara et al. (1965) and Ueda et al. (1967). These authors have found that infusing home water from the spawning site into the nostril of returning adult chinook and silver salmon, evokes a characteristic (in amplitude and pattern) electrical response in the olfactory bulb. They reported that waters from completely different drainages would not evoke the home water response. However, in a few tests they found that waters bypassed by the homing salmon yielded olfactory bulbar electrical responses that were inferior to the home water response. For this reason, Ueda et al. (1967) made the tentative proposal that ascending salmon may move in the direction which provokes the larger

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olfactory bulbar response. Implied in this idea is that the migration stops when further movement beyond the "remembered" home water yields a smaller evoked nervous response.

Since the suggestions of Ueda et al. (1967) were tentative and based on a relatively small number of geographical sources of tested waters, we elected to test them under better experimental conditions. Improvement in conditions was achieved by setting up the electrophysiological equipment at the three hatcheries into which the tested fish migrated so that the salmon did not need to be transported to the laboratory; also, collections of the tested waters were made from a larger number of points along the route of migration, or beyond it.

It was reasoned that in these experiments, if the hypothesis of Ueda et al. is correct, the salmon are probably homing on an increasing concentration of a single olfactory clue. If the hypothesis were born out, the various water samples taken along the migratory route should be increasingly stimulatory as their distance from the spawning site was decreased.

MATERIALS AND METHODS

Since preliminary work had shown that the olfactory quality of natural waters deteriorates gradually at room temperatures, test water collections were made almost always on the day of use and water samples were kept at 4–6 C until used. The sites for collection of water samples are indicated on the accompanying maps (Fig. 2, 4, 6).

All salmon used were selected from the relatively small size range, since larger specimens could not be accommodated in the available equipment; they weighed between 3 and 12 kg. Seven chinook salmon (*Oncorhynchus tshawytscha*) were tested at the end of their migration to the University of Washington College of Fisheries pond, on the campus of the University (October 18–20); eight silver salmon (*Oncorhynchus kisutch*) were tested at the State of Washington hatchery on the Issaquah River, near Issaquah, Wash. (November 8–15); and five coho salmon were tested at the State of Washington hatchery on Soos Creek, near Auburn, Wash. (December 4–11).

For testing of the olfactory responses the techniques used were essentially as described for goldfish by Oshima and Gorbman (1966) and for salmon by Oshima et al. (1969). Briefly, the procedure involved light anesthesia in tricaine methanesulphonate (M.S. 222) and muscular immobilization with gallamine triethiodide (Flaxedil). The salmon was placed in a molded retainer and the gills irrigated with flowing water for respiration. Rapid dissection exposed the dorsal side of the brain, and the bipolar stainless steel electrode was placed on the olfactory bulb to record its electrical output. Water samples from each tested site were infused in random order into the nostril through a fine polyethylene tube at a constant rate for 15 sec. A distilled water rinse followed each tested natural water. Each natural water was infused for at least three separate tests.

The electrical electroencephalographic (EEG) output of the olfactory bulb was amplified (Grass 5P5) and recorded on a Grass Model D polygraph. By also running the amplified EEG through an electronic integrating circuit (Grass 5P3) another record was obtained which summated the total electrical activity as a function of time. Since the integrated record appeared as a single line displaced from the base line, an arbitrary quantitative value was obtained for each olfactory EEG by measuring the area under the first 10-sec period of the evoked olfactory response (for example, see Fig. 2).

RESULTS

DILUTION TESTS

A first series of experiments was designed to determine the degree of dilution of home water that would still evoke an EEG response in the olfactory bulb. Several such experiments were done, and one is illustrated in Fig. 1. Generally, a recognizable response could no longer be recorded when the dilution with distilled water was between 10:1 and 20:1. Soos Creek contributes less than 10% of the volume of the Green River below the junction of these two streams (Fig. 2). Accordingly, this series of tests would indicate that if olfactory responses are obtained from downstream points on the Green River they cannot be accounted for on the basis of diluted Soos Creek home water.

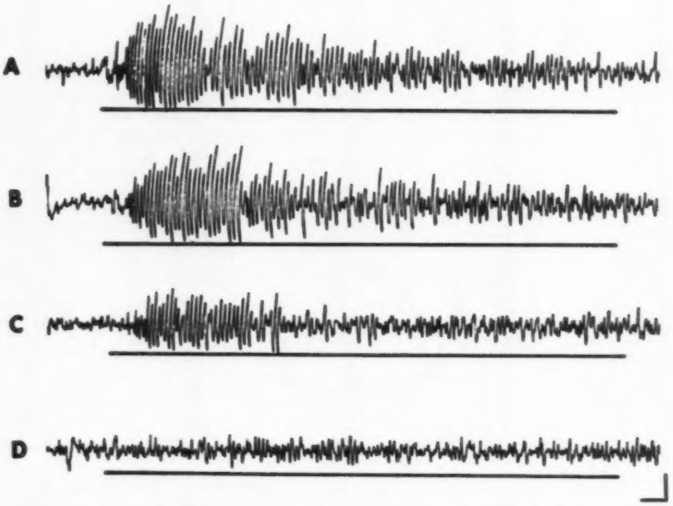

FIG. 1. Bulbar EEG responses of coho salmon at Soos Creek to their home water and to various dilutions of home water with distilled water. A, undiluted home water; B, 50%; C, 25%; and D, 5%. Solid line indicates the period of nasal infusion. Calibration: horizontal, 1 sec; vertical, 50 μv.

Green River – Soos Creek Tests

As Fig. 2 indicates, tested water samples were taken from the spawning area on Soos Creek (*a*), six points extending from just below the confluence of the Green River with Soos Creek (*b*) successively farther (*c*, *d*, *e*, *f*) toward the tidal brackish river mouth, and from a point 2 miles above the confluence of Soos Creek and Green River (*h*). The bulbar responses to water samples taken at each of these points are shown in Fig. 3 and mean integrated bulbar EEG responses are analyzed in Table 1. It is clear in these tests that the electrical olfactory bulbar response is fairly uniform for samples taken at various points along the brackish to freshwater route. The tests of saline water samples (sample area *g*, Fig. 2) and of water taken upstream of Soos Creek (area *h*, Fig. 2) yielded responses which did not differ significantly from the response to the spawning area home water (sample station *a*, Fig. 2). Hence,

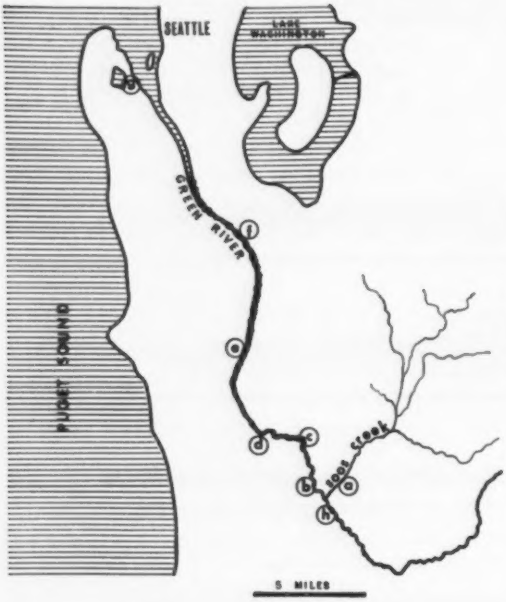

FIG. 2. Freshwater migratory route for Soos Creek coho salmon. The migration terminates at point *a* where the Washington State Green River Fish Hatchery is located. Encircled letters indicate sites of collection of water samples used in tests for evocation of olfactory bulbar electrical responses. Further descriptions in Table 1 and text.

it is clear that an intense bulbar response is produced by Green River water even before the addition to it of the Soos Creek flow. Water taken from a spring near the spawning area on Soos Creek evoked a response clearly distinguishable (26.4% of home water response) from those produced by migratory route waters. Skykomish Creek water (a completely unrelated drainage) also evoked a significantly lower electrical response (73.7 ± 9.1% of home water response; $P < 0.025$ vs. home water).

UNIVERSITY OF WASHINGTON COLLEGE OF FISHERIES TESTS

Chinook salmon that had homed to the University of Washington College of Fisheries pond (A, Fig. 4) were tested for responses to water samples collected at points along their migratory route and one point beyond it. Samples were from tidal brackish Shilshole Bay and freshwater loci indicated in Fig. 4. Representative bulbar EEG responses to these waters and their mean integrated values are illustrated in Fig. 5 and analyzed in Table 2. Freshwater samples (C–H) along the migratory route evoked variable bulbar EEG responses which were lower than the home water response ($P < 0.005$–$P < 0.001$ vs. home water). However, there was no clear direction of variation that could be taken as a gradient. Tidal brackish water samples induced significantly higher electrical bulbar responses than several freshwater samples from points D and F in Fig. 4 ($P < 0.05$). Among freshwater samples only water taken from Wolf Bay, Lake Washington, evoked a high enough response to be indistinguishable statistically from the home water response.

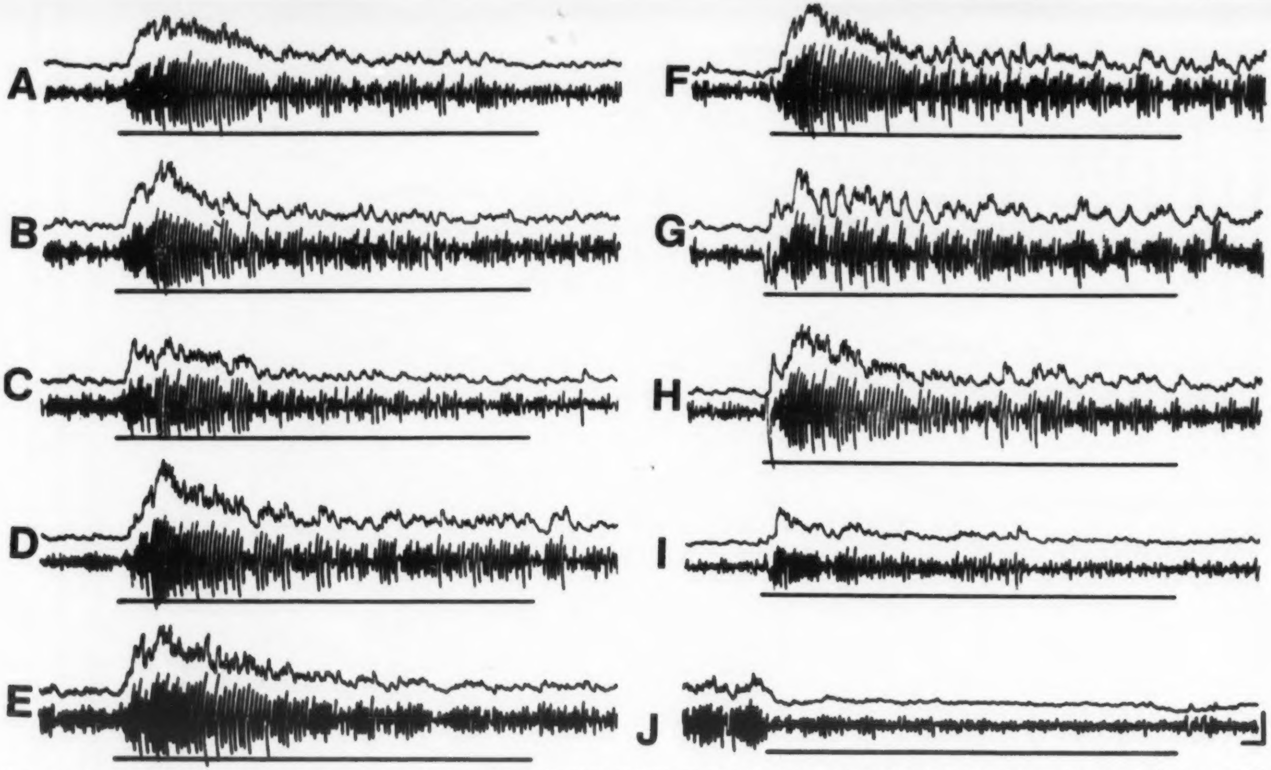

FIG. 3. Bulbar EEG responses during nasal infusion of freshly obtained water samples from the specific locations shown in Fig. 2. Response I is to spring water obtained near the hatchery; J is the "depression" response obtained by nasal infusion of distilled water. Solid line indicates infusion period. Calibration: horizontal, 1 sec: vertical, 50 μv.

TABLE 1. Green River – Soos Creek test of coho salmon (December 4–11, 1967).

	Test water	No. of fish	% of home water response
a	Soos Cr. (home water)	5	100.00
	Skykomish Cr.	4	73.7±9.1[a]
	Issaquah Cr.	4	101.6±6.9
g	West Water Way	4	112.3±13.8
f	Pacific Hwy. Junction	4	91.2±4.2
e	Tukwila	5	90.4±8.6
d	Kent	4	99.2±11.5
c	2 miles before Auburn Bridge	5	103.6±13.0
b	Auburn Bridge	3	102.7±14.5
h	2 miles upstream	4	92.8±10.7
	Spring water	3	26.4±6.1[b]

[a] $P < 0.025$.
[b] $P < 0.001$.

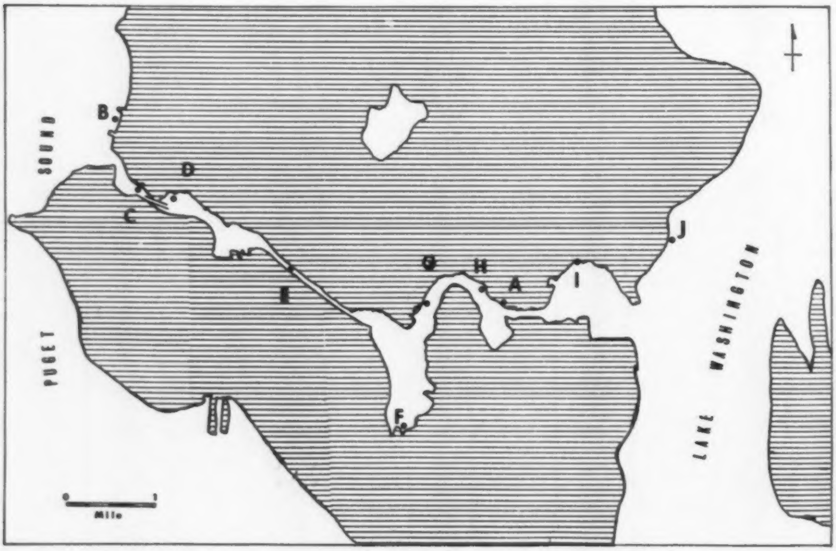

FIG. 4. Freshwater migratory route for University of Washington College of Fisheries chinook salmon. The migration terminates at point A where the University of Washington College of Fisheries pond is located. Letters along the route indicate sites of collection of water samples. Detailed description in Table 2.

ISSAQUAH CREEK HATCHERY TESTS

The migratory route for Issaquah Creek salmon (silver) is the longest of the three we studied. Loci for collection of the water samples are shown on Fig. 6 and the results of tests are summarized in Table 3.

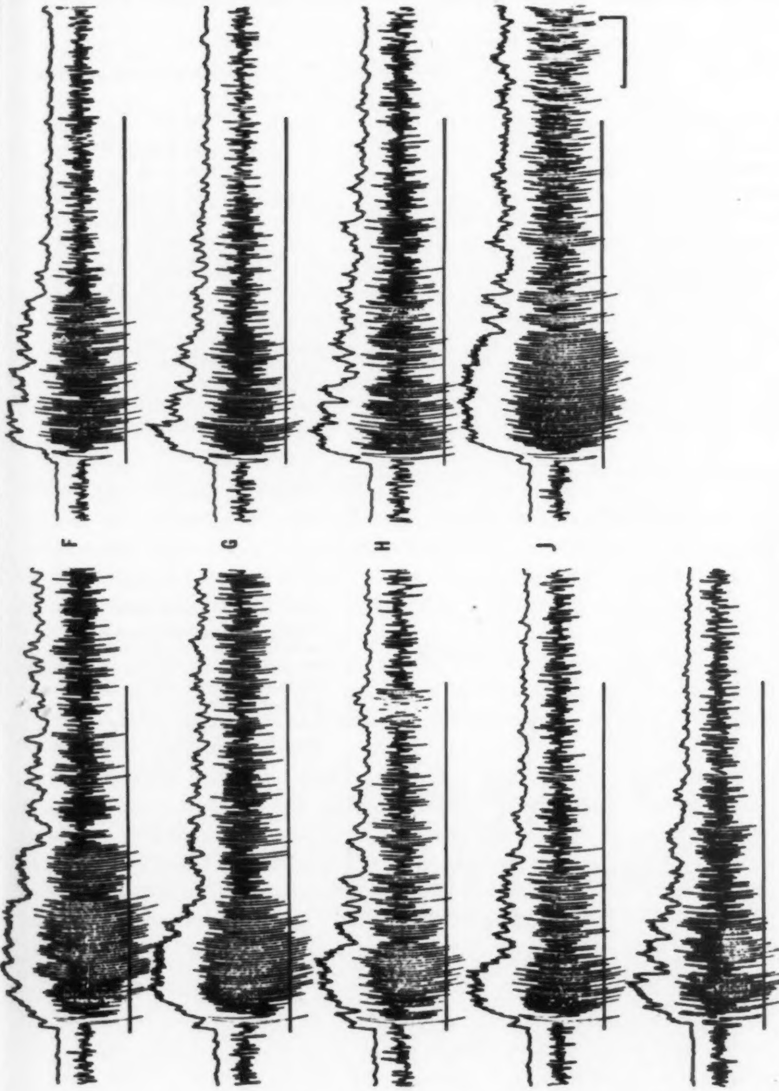

FIG. 5. Bulbar EEG responses during nasal infusion of freshly obtained water samples from specific locations shown in Fig. 4. Letters at left side correspond to sites of water collection shown in Fig. 4 and Table 2. Calibration: horizontal, 5 sec; vertical, 50 μV.

In the Issaquah experiments there was no evidence of an olfactory gradient of responses. However, as in the University of Washington series, most of the nonspawning-area water responses were inferior to the home water response. Highest responsiveness was to water from several points in Issaquah Creek,

TABLE 2. University of Washington College of Fisheries test of chinook salmon (October 18–20, 1967).

	Test water	No. of fish	% of home water response
A	Home pond	7	100.00
	Soos Cr.	6	53.9±10.7[a]
B	Shilshole Bay	6	76.1±6.0
C	Before locks	5	87.2±11.2
D	After locks	5	54.3±7.2[b]
E	Washington Ship Canal	5	61.8±7.6
F	L. Union, south end	7	53.5±7.1[b]
G	L. Union, north end	5	61.2±9.2
H	Portage Bay	6	64.8±10.3
I	Union Bay	4	70.9±7.4
J	L. Washington, Wolf Bay	6	85.8±11.8

[a] $P < 0.001$ (vs. home water).
[b] $P < 0.05$ (vs. B).

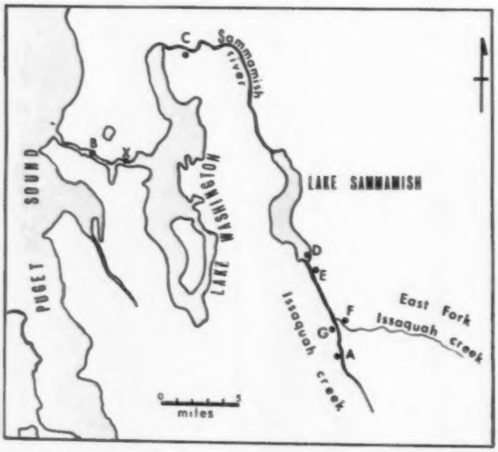

FIG. 6. Freshwater migratory route for Issaquah Creek silver salmon. The migration terminates at point A where the Washington State Issaquah Fish Hatchery is located. Letters along the route indicate sites of collection of water samples. Detailed description in Table 3.

close to the spawning area (A, Fig. 6). It is of particular interest that water from the East Fork of Issaquah, a tributary that joins Issaquah Creek below the hatchery, was among the least stimulatory of the samples tested, about equal in olfactory value to Soos Creek, which is unrelated to Issaquah Creek. These results would seem to be important for interpretation of the data of Wisby and Hasler (1954) which are described from work with salmon at this same locus.

DISCUSSION

The near equality of the bulbar responses to water taken from sites bypassed by upstream migrating salmon (Table 1–3) suggests that they do not

TABLE 3. Issaquah Creek Hatchery test of silver salmon (November 8–15, 1967).

	Test water	No. of fish	% of home water response
A	Home water	8	100.00
	Soos Cr.	4	63.1±10.4[a]
X	Univ. Wash. Fisheries Pond	6	74.5±6.5
B	Ship Canal	8	74.1±10.0[b]
C	Wayne Slough (Sammamish R.)	8	74.7±11.6[c]
D	L. Sammamish	8	55.6±9.2[d]
E	Issaquah R.-L.	3	93.6±12.1
F	East Fork, Issaquah Cr.	8	63.4±4.8[e]
G	Issaquah Cr.	8	90.5±7.7

[a] $P < 0.001$ (vs. Soos Creek water).
[b] $P < 0.025$ (vs. home water).
[c] $P < 0.05$ (vs. home water).
[d] $P < 0.001$ (vs. home water).
[e] $P < 0.01$ (vs. G).

follow an increasing gradient of a single type of odorant, which steadily increases the intensity of the bulbar responses. However, home water responses were much higher than most downstream water samples in the University of Washington chinook salmon and in Issaquah silver salmon. On the basis of more limited observations, Ueda et al. (1967) concluded that the olfactory bulbar response was clearly discriminatory at stream junctions and at downstream vs. home water loci, and that these olfactory bulbar responses corresponded to the actual behavioral "choice" of route made. Our data based on tests from a larger sequence of sources of tested waters do not permit the same unqualified conclusion.

If isolated instances were considered, then our data contain some clear illustrations that these fish do discriminate at stream junctions and between bypassed and final points in the migratory route (e.g., E and F, Fig. 6). However, on the basis of bulbar EEG responses we mostly failed to obtain a correlation between the relative intensity of the bulbar EEG response and actual behavioral performance. If the original thesis, as outlined by Ueda et al. (1967) is not sustained, then the alternative may be that the salmon are following a succession of olfactory clues. Interpreted on the basis of the quantitative data alone, as summarized in the three tables, it would seem that the salmon are experiencing a series of olfactory stimuli of approximately equal "value" to the olfactory bulb, and each stimulus is characteristic of the precise locus at which it occurs. In other words, in the olfactory sense, the salmon would appear to remember the odor of specific loci separately, and probably in a particular order, rather than a single odor from a level of infinite dilution to maximum concentration.

It must be remembered that our examination of the bulbar EEG is limited to measuring its relative intensity. The recorded pattern of the response does not reveal any qualitative features which are consistent and limited to one type of olfactory stimulation. Accordingly, bulbar EEG offers only a limited opportunity for us to judge qualitative elements in the olfactory stimulus other than those aspects which are reflected in the intensity of the response. A part of the intensity of the response is related to the intensity of the stimulus (Fig. 1). However, part of the olfactory bulbar response intensity and some of its qualitative features are regulated by centrifugal influences that reach the olfactory bulb through the olfactory tract (Hara and Gorbman, 1967; Döving, 1966a, b; Döving and Gemne, 1966; Takagi, 1962). It may be supposed that, to some degree, the centrifugal influences on the olfactory bulb may represent reflected "analytical" nervous activity related to "olfactory memory." However, this is an area that requires further study. It is mentioned here only to outline the complex character of olfactory bulbar electrical responses. It is clear that although the bulbar response to natural waters is the only tool available for experimental application to the problem of olfactory guidance of salmon, it is a tool that yields data the significance of which is mostly cryptic.

The justification for use of electrical bulbar response in continued study of olfactory guidance for migration of salmon resides in the invariable intense response made to home water relative to the responses to water taken from other drainage areas. Since in tests of several homing populations it is always the home water that evokes the characteristic high amplitude response, the bulbar response reflects not only the intensity of the olfactory stimulus, but also in part a specific discriminative element (based, presumably, on prior experience, imprinting, and olfactory memory). It should be mentioned here that the Green River especially acquires additional variable olfactory elements due largely to pollution. These additions, however, had no marked effect on the bulbar response. As pointed out by Hasler (1966), it seems reasonable that the most adaptively useful odors on which salmon depend for homing clues are those which are stable from year to year for a given drainage system.

An apparently significant result obtained in these experiments was in tests of water samples at the East Fork juncture of Issaquah Creek (F and G, Fig. 6). These two points had been selected for olfactory occlusion experiments by Wisby and Hasler about 15 years ago (Wisby and Hasler, 1954; Hasler, 1966). They found that fish with olfactory occlusion were unable to repeat their original choice at this stream juncture, thus furnishing experimental support for olfactory homing theory. Our data, which showed clearly that olfactory response to East Fork water was much smaller than to Issaquah Creek water, appears to support electrophysiologically their results.

ACKNOWLEDGMENTS

We are very grateful to Dr L. R. Donaldson, Mr S. B. Fallert, and Mr Roy Engel for providing space and equipment and for the supply of spawning salmon. This work was aided by a research grant NB04881 from the National Institutes of Health, U.S. Public Health Service.

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Composition and Horizontal Distribution of Crustacean Plankton in Lake Ontario

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Patalas, K. 1969. Composition and horizontal distribution of crustacean plankton in Lake Ontario. J. Fish. Res. Bd. Canada 26: 2135–2164.

The horizontal distribution of planktonic crustaceans was determined in Lake Ontario at monthly intervals from June to October 1967, based on net hauls from 50 to 0 m at 32–62 stations. A separate study conducted over a 24-hr period at one station showed that on the average 90% of the zooplankters occupied the 0–50-m stratum through which the net was hauled. Eleven species of copepods and 11 species of cladocerans were found. The most abundant forms were *Cyclops bicuspidatus thomasi*, *Daphnia retrocurva*, *Bosmina longirostris*, *Bosmina coregoni coregoni*, *Tropocyclops prasinus mexicanus*, and *Ceriodaphnia lacustris*. Most species appeared in the collections during June–July in the eastern part of the lake with abundance patterns later moving westward, contrary to the general movement of wind and water. At the time of maximal population density there was a strong positive correlation between zooplankton abundance and heat content of the water column from 0 to 25 m. The eastern part of the lake averaged 1.7 times more specimens/cm^2 than the western part of the lake. Upwelling was responsible for low numbers of zooplankters along the northwestern shore. Abundance patterns similar to generalized current flows were observed in the northern and southern parts of the lake. Flow from the Niagara River was associated with increased abundance of zooplankters in spring and summer, but decreased abundance in autumn. A detailed study of the abundance and distribution of zooplankton offshore from Toronto Harbour showed that harbour water does influence zooplanktonic populations in the vicinity. Temperature and depth of the epilimnion as influenced by the general direction of wind motion were key factors in understanding the zooplanktonic community of Lake Ontario.

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INTRODUCTION

Lake Ontario, the lowermost of the St. Lawrence Great Lakes, has a surface area of 18,760 km^2, a land drainage area of 67,300 km^2, a maximum depth of 237 m, and a mean depth of 91.4 m. The human population living in the basin, 6.1 million in 1966, has grown exponentially since the time of early settlement. Due to factors related to population growth in both the Lake Ontario drainage basin and the Great Lakes basin as a whole, a number of pronounced changes in the chemistry and biology of the lake have occurred during the past 60 years. These and prior limnological studies have been summarized by Beeton (1965) and Davis (1966). The present investigation was undertaken to determine the species and abundance of crustacean zooplankton on a lake-wide basis as part of a concerted effort by the Fisheries Research Board of Canada to characterize the biological communities of the Great Lakes relative to natural and cultural eutrophication processes.

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Among the several factors influencing the horizontal distribution of zooplankton, those of temperature, morphometry of the basin, exposure to wind, location of influents and effluents, and the chemistry of water masses play predominant roles. For many lakes it is common to assume that individual species are homogeneously distributed under a unit area of lake surface, accepting 10–20% variation as insignificant (Ruttner, 1963). This has, for example, been shown to be the case for Grosser Plönersee (Naber, 1933), 30 km^2 in area; Lake Charzykowo (Patalas, 1954), 13.7 km^2 in area; and Lake Erken (Nauwerck, 1963), 22.8 km^2 in area. Some lakes show much greater heterogeneity: Lago Maggiore (Tonolli, 1949, 1958), 212 km^2 in area; and Lake Skärshult (Berzins, 1958), 0.36 km^2 in area. Ricker (1937) showed for Cultus Lake (6.3 km^2) and Langford (1938) for Lake Nipissing (135 km^2) that some species of zooplankton were randomly distributed, but others not. It was thus considered essential to determine the horizontal variation in abundance of zooplankton in Lake Ontario as a requisite for the development of statistically significant sampling programs and as a starting point for future investigations on trophic-dynamic aspects of the zooplankton community. This is apparently the first detailed study of zooplankton distribution in such a large lake.

METHODS

Monthly samples were collected from June to October 1967, at 32 stations (62 in July) distributed over the entire lake. Sixty per cent of the stations were deeper than 50 m, 20% in the range of 25–50 m, and 20% in the range of 14–24 m. The duration of each cruise was 3-4 days, thus yielding semi-synoptic data. At each station a vertical haul was taken with a Wisconsin-type plankton net (pore opening 77 μ, mouth diameter 25 cm) from a depth of 50 m to the surface, or from just above the bottom to the surface at stations shallower than 50 m. The net was hauled at a rate of 0.3–0.5 m/sec. The samples were preserved in 2% formaldehyde. At least 200 specimens were counted and identified from each sample. Depending on the densities of plankton 1/100–1/500 of the sample was examined microscopically. Zooplankton abundance was expressed as the number of specimens/cm^2 of lake area, assuming that the filtration efficiency of the net was 100%. The data should not be interpreted as true densities, but rather as relative abundance values proportional to densities since the true efficiency probably varies from 40 to 50% (Rawson, 1956).

Since the purpose of the cruises was to obtain semi-synoptic information for several independent investigators, the sampling operations had to be carried out at whatever time of the day or night the ship reached a station. An analysis of the zooplankton data showed no significant relation between the abundance of most species and the time of sampling, except in the case of *Limnocalanus macrurus*. Figure 1 shows the abundance of *L. macrurus* in 0–50-m hauls as a function of time of day for all samples from each of two cruises. It is apparent that appreciable numbers were only collected during the night. For this reason no attempt was made to calculate abundance.

Special samples were taken on September 12–13, 1968, to elucidate certain questions connected with data collected in 1967, notably the vertical distribution of zooplankton relative to the standard 0–50-m hauls at different times of the day and night. The ship was positioned near the centre of the lake at 77°25′W, 43°40′N, where the depth of water was approximately 100 m. A series of samples was collected every 3 hr using five 6-litre van Dorn bottles arranged at 5-m depth intervals within each of four layers: 0–25 m, 25–50 m, 50–75 m, and 75–100 m. The contents of the van Dorn bottles within each layer were combined and filtered through silk gauze with a pore diameter of 77 μ.

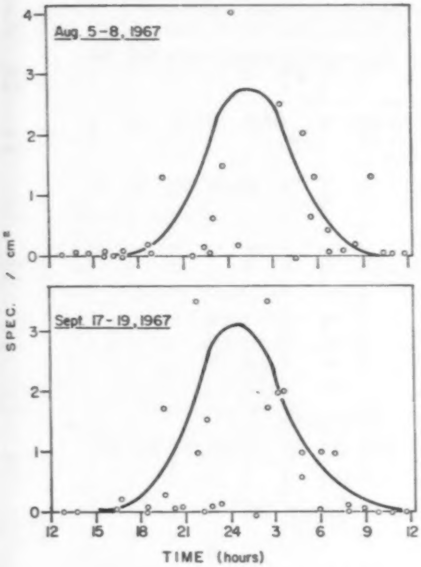

FIG. 1. Relation between the time of sampling and the density of *Limnocalanus macrurus* in vertical hauls.

RESULTS

SPECIES AND VERTICAL DISTRIBUTION

Twenty-two species of crustaceans were identified in the June to October collections, 11 copepods and 11 cladocerans. These are listed in Table 1 together with comparative lake-wide abundance values and previous records of their occurrence in Lake Ontario and adjacent bays. Of the 22 species reported in this study only 7 had been previously recorded from Lake Ontario proper (14 from Lake Ontario plus the three isolated bays listed in Table 1). The most abundant copepods found in this study were *Cyclops bicuspidatus thomasi* and *Tropocyclops prasinus mexicanus*. The most abundant cladocerans were *Daphnia retrocurva*, *Bosmina longirostris*, *B. coregoni coregoni*, and *Ceriodaphnia lacustris*. *Epischura lacustris* and *Senecella calanoides*, found by Pritchard (1931) in the stomach of a single cisco (*Leuciscus kiyi*) were not found in the present study in spite of the intensive sampling and special series of deep hauls, nor by Robertson (1966). Mr M. F. P. Michalski (personal communication), however, has occasionally found single specimens of *Epischura lacustris* in nearshore waters from the eastern part of the lake.

Figure 2 shows the results obtained from the study of vertical distribution in September 1968. It can be seen that for most species 90% of the population was restricted to the uppermost 50 m. The two notable exceptions were *Limnocalanus macrurus* and *Diaptomus sicilis*. More than 50% of their populations occurred in the 50–100-m depth interval. There was also a suggestion that the three diaptomids had different patterns of vertical distribution: *Diaptomus*

TABLE 1. Species composition of crustacean plankton in Lake Ontario with references to previous records for Lake Ontario (LO), Irondequoit Bay (IB), Bay of Quinte (BQ), and Sodus Bay (SB).

Species	Avg abundance in the whole lake for the period June–October		Previous records
	Specimens/cm^2	%	
	Copepoda		
Limnocalanus macrurus Sars	0.6	0.18	Pritchard, 1931 (LO)
Eurytemora affinis (Poppe)	2.5	0.76	Anderson & Clayton, 1959 (LO)
Diaptomus minutus Lilljeborg	2.8	0.85	Robertson, 1966 (LO)
Diaptomus oregonensis Lilljeborg	2.3	0.70	Robertson, 1966 (LO)
Diaptomus sicilis Forbes	2.2	0.67	Clemens and Bigelow, 1922 (LO) Robertson, 1966 (LO)
Diaptomus siciloides Lilljeborg	<0.01	<0.01	Tressler et al., 1953 (IB) Robertson, 1966 (LO)
Diaptomus pallidus Herrick	<0.01	<0.01	
Cyclops bicuspidatus thomasi Forbes	153.0	46.3	Tressler et al., 1953 (IB)
Cyclops vernalis Fischer	0.2	0.06	
Tropocyclops prasinus mexicanus Kiefer	36.0	10.9	
Mesocyclops edax (Forbes)	0.4	0.12	Tressler et al., 1953 (IB)
	Cladocera		
Daphnia retrocurva Forbes	37.0	11.2	Brooks, 1957 (IB)
Daphnia longiremis Sars	0.4	0.12	
Daphnia galeata mendotae Birge	0.01	0.01	Brooks, 1957 (IB)
Ceriodaphnia lacustris Birge	5.4	1.63	
Bosmina longirostris (O. F. Müller)	77.0	23.3	Hart, 1930 (BQ)
Bosmina coregoni coregoni Baird	10.0	3.03	
Chydorus sphaericus (O. F. Müller)	0.1	0.03	
Holopedium gibberum Zaddach	0.1	0.03	Hart, 1930 (BQ)
Diaphanosoma leuchtenbergianum Fischer	0.03	0.01	
Leptodora kindtii (Focke)	0.16	0.05	Pritchard, 1931 (LO)
Polyphemus pediculus (Linne)	0.03	0.01	Hart, 1930 (BQ)
	Σ 330.25	100.0	
	Species not found in present study		
Epischura lacustris Forbes		0.0	Pritchard, 1931 (LO)
Senecella calanoides Juday		0.0	Pritchard, 1931 (LO)
Diaptomus reighardi Marsh		0.0	Marsh, 1901 (SB)

minutus was largely confined to the 0–25-m depth interval; *D. oregonensis* was distributed more or less evenly between the 0–25 and 25–50-m depth intervals; and *D. sicilis* occupied all four depth intervals. These observations agree with those of Langford (1938) for Lake Nipissing and Rigler and Langford (1967) for Round, Mill, and Clark lakes in Ontario.

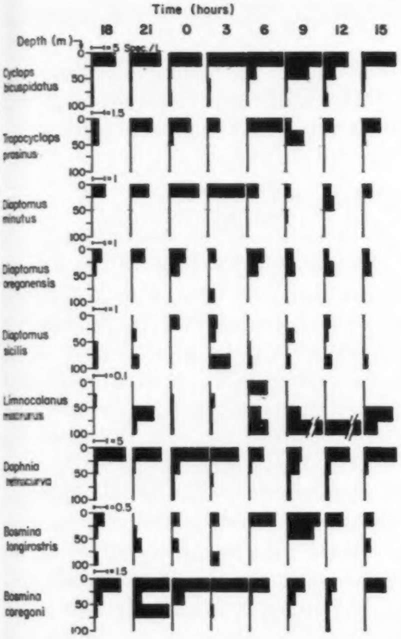

FIG. 2. Changes in the vertical distribution of plankters in 24-hr period. (September 12–13, 1968, near the centre of the lake.)

The dominant species in the September 1968 samples were *C. bicuspidatus thomasi*, *B. longirostris*, and *D. retrocurva*, all of which occurred predominantly in the 0–50-m layer. *Limnocalanus macrurus* and *D. sicilis*, the two species not largely confined to the 0–50-m interval, formed only a minor fraction of the total crustacean plankton. In terms of all samples over the 24-hr period, 73% of the specimens were taken from the 0–25-m layer and 90% from the 0–50-m layer. If these results are representative of the June–October sampling period, it can be inferred that the routine plankton hauls give a reliable picture of zooplanktonic abundance in the 0–100-m depth interval.

For some species the abundance per unit area in the 0–100-m depth interval varied markedly during the 24-hr period (see Fig. 2). This is probably a statistical artifact resulting from the wide intervals (5 m) between the van Dorn bottles, and other factors. In spite of this deficiency the general trend of the vertical migrations for different species can be seen in Fig. 2. Most species migrated within the 0–50-m interval. At night (2100–0600 hr) crustaceans were largely restricted to the 0–25-m interval, whereas during the day (especially 0900–1500 hr) part of the populations moved into the 25–50-m layer. This pattern was typical of *C. bicuspidatus thomasi*, *T. prasinus mexicanus*, *D. minutus*, *D. oregonensis*, and, to a certain extent, also of *D. retrocurva* and *B. coregoni coregoni*. The migrations of *L. macrurus* covered a much greater vertical distance. During the day this species was concentrated below 50 m depth, and only during the night (1800–0600 hr) did part of the population migrate above that depth.

Distributional maps showing the abundance of various species from June to October (1967) are given in Fig. 3–13, based on the analysis of vertical plankton hauls. These are discussed below separately for each species.

CLADOCERA

Bosmina longirostris (Fig. 3) was the most abundant crustacean in the summer plankton of Lake Ontario. It appeared first in June in the eastern part of the lake centering around Henderson Bay, and in an area in the west centering around the mouth of the Niagara River. In July the species occurred throughout the lake, with average numbers/cm^2 in the eastern part about 10 times higher than those in the central and western parts. Around Henderson Bay and in the vicinity of Oswego more than 500 specimens/cm^2 were found. Abundance decreased gradually toward the west to minimum values of less than 10 specimens/cm^2 along the northwest shore (Toronto region). An area of relatively high abundance (over 100 specimens/cm^2) occurred northeast of the Niagara River. In August and September the distributional patterns were almost the reverse of that in July: maximum densities occurred in the western part of the lake with abundance lowest in the east. A relatively uniform distribution over the lake was noted in October, except for two areas: the southern shore, where over 100 specimens/cm^2 occurred in the area of Rochester–Oswego; and the northern shore where there were fewer than 10 specimens/cm^2.

Bosmina coregoni coregoni (Fig. 4) had a distributional pattern markedly different from that of the more abundant *B. longirostris*, in all months other than June. During July and August, for example, abundance increased gradually in the east, but samples from the whole northern and central area of the lake were devoid of *B. c. coregoni*. In September–October the species was present in the central area of the lake, with the densities in the western end extremely low. In many of the latter samples the species was absent.

Ceriodaphnia lacustris (Fig. 5) in June occurred only as a single specimen in one sample taken from Henderson Bay in the extreme eastern end of the lake. In July the density was over 10 specimens/cm^2 in the vicinity of Henderson Bay. By August the species occurred over the whole eastern part of the lake with densities in excess of 100 specimens/cm^2. No specimens were detected in the central and western parts of the lake in August. By September the distributional pattern had changed dramatically with maximum densities in the western end, and an almost complete disappearance from the eastern end of the lake, i.e. similar to the shift in pattern observed for *B. longirostris*. In the October samples *Ceriodaphnia* was either rare or lacking throughout most of the lake with the exception of the area offshore from Rochester–Oswego.

Daphnia retrocurva (Fig. 6) in June was present in the northeastern part of the lake near the St. Lawrence River. Single specimens were also found

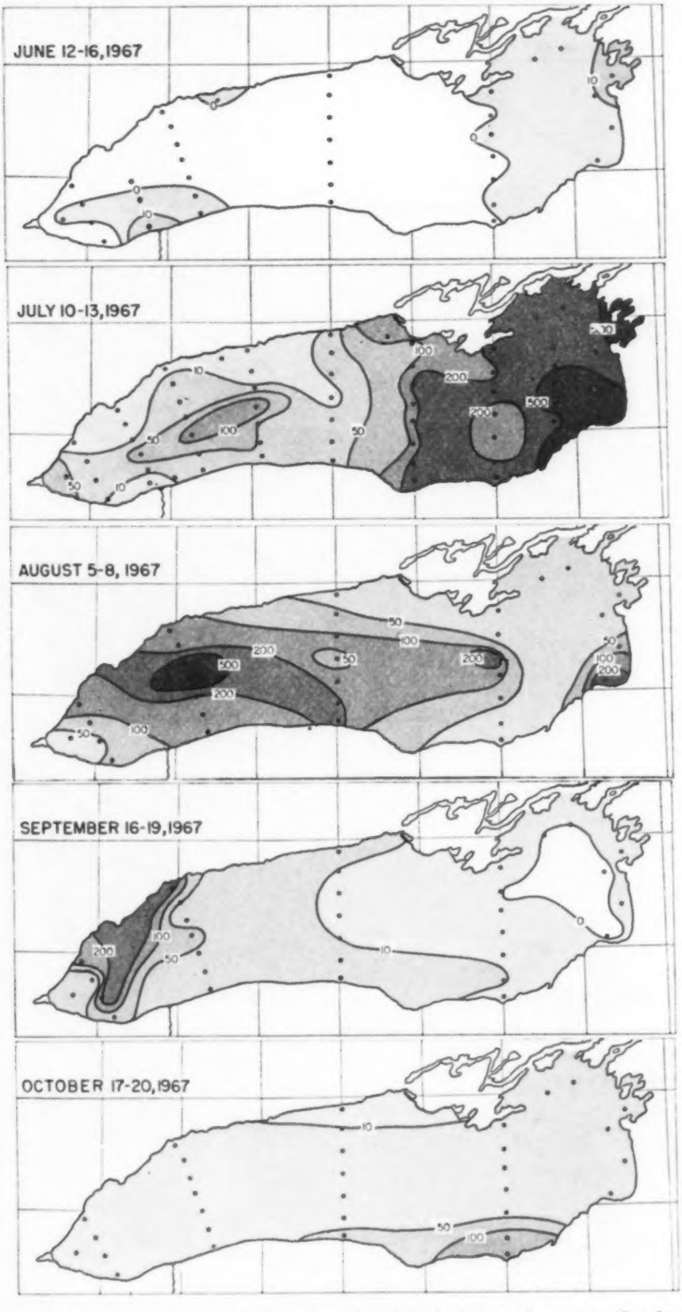

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FIG. 3. Distribution of *Bosmina longirostris* from June to October, specimens/cm².

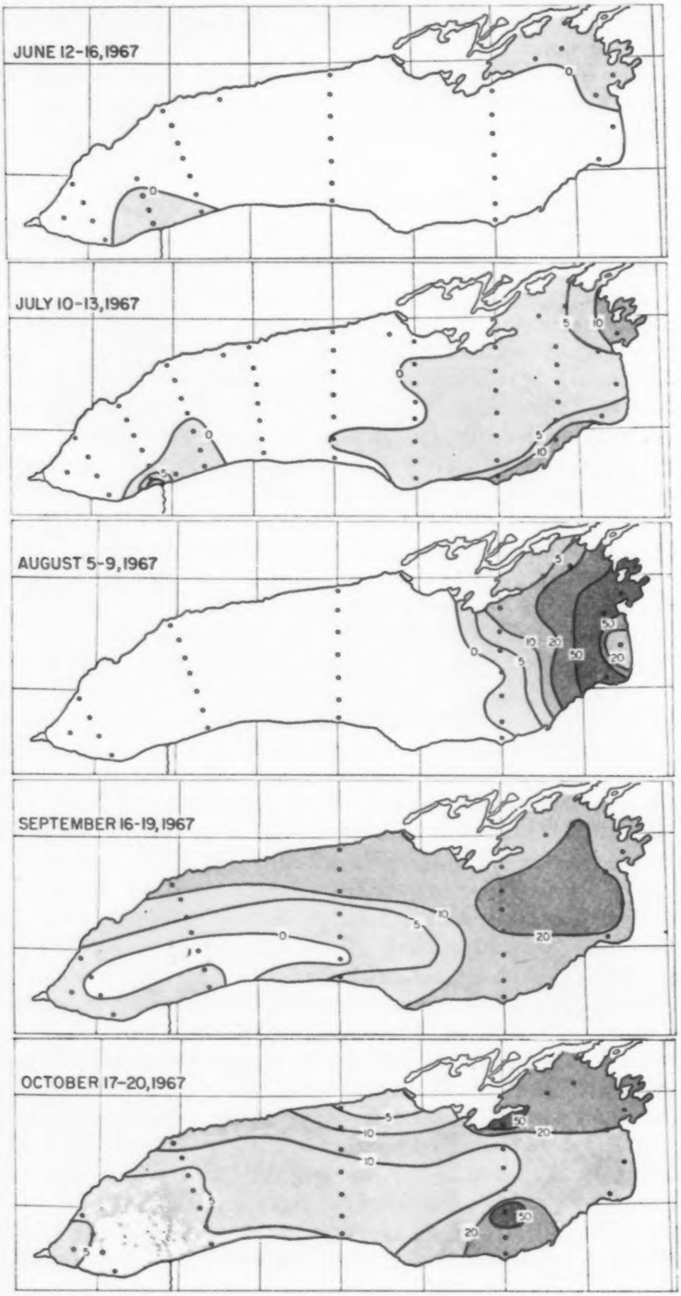
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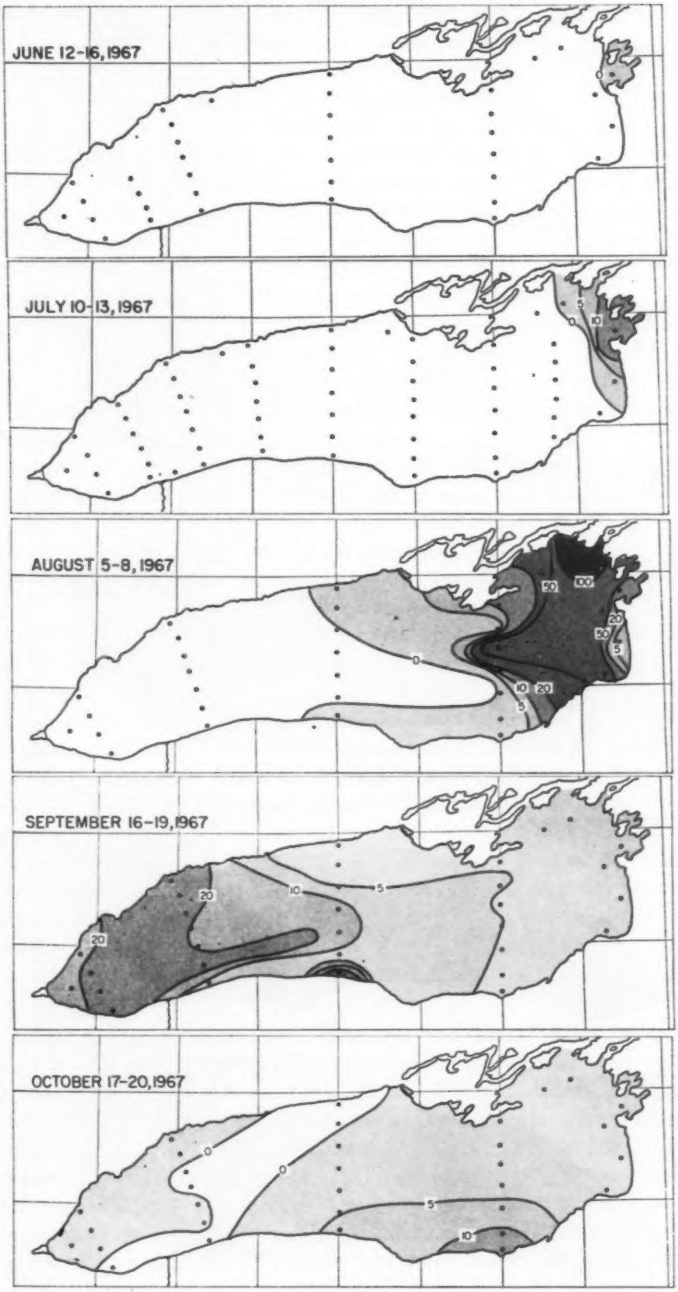
FIG. 4. Distribution of *Bosmina coregoni coregoni* from June to October, specimens/cm^2.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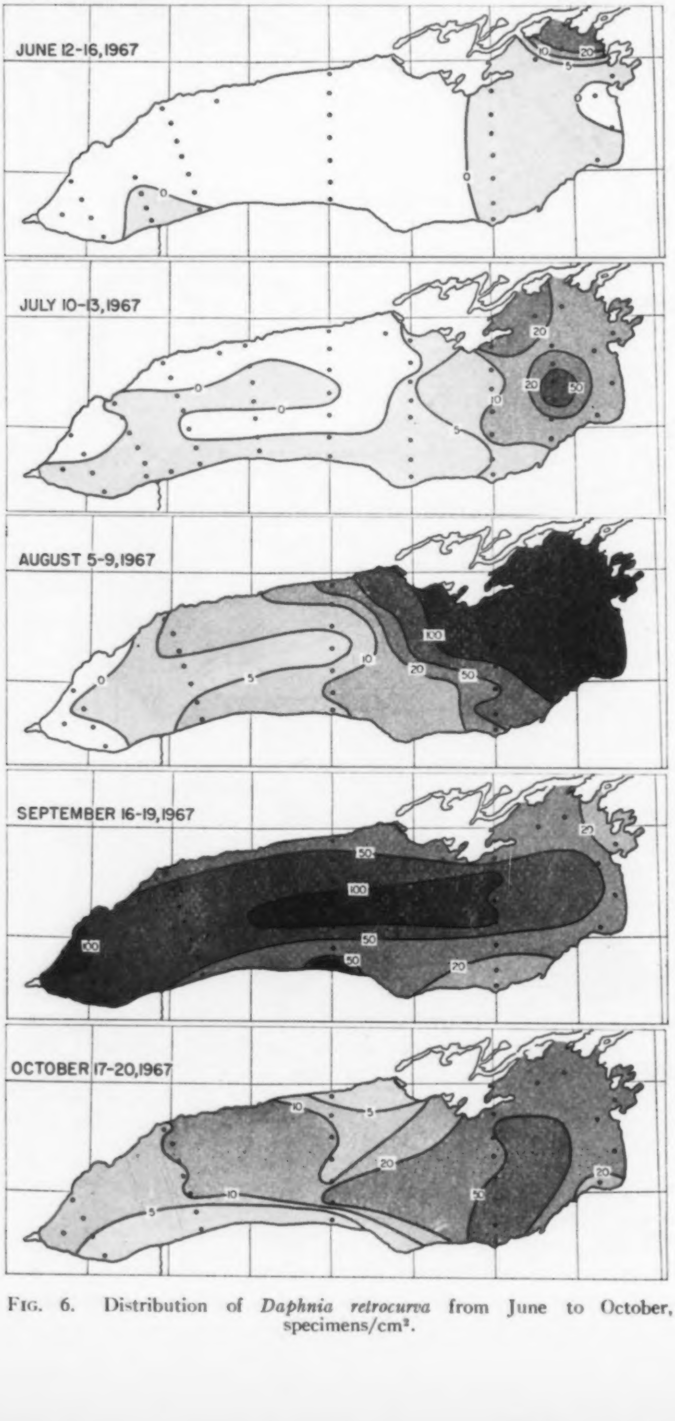
0
0
5
10

FIG. 5. Distribution of *Ceriodaphnia lacustris* from June to October, specimens/cm^2.


JUNE 12-16, 1967
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10
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50
100
SEPTEMBER 16-19, 1967
20
50
100
OCTOBER 17-20, 1967
5
10
20
50

FIG. 6. Distribution of *Daphnia retrocurva* from June to October, specimens/cm².

in several vertical hauls taken near the mouth of the Niagara River. By July the species was distributed throughout the eastern part of the lake, reaching a maximum density there in August over 200 specimens/cm^2 with an extension of the population into the central part. *Daphnia retrocurva* was not encountered in any hauls from the western end taken in July and August; however, by September it was distributed over the entire lake with maximum density (in excess of 100 specimens/cm^2) in the central part. The density of *Daphnia retrocurva* in October samples from the central and western parts was about $\frac{1}{10}$ of the September values, whereas the abundance in the eastern end was at about the same level as in September.

Daphnia longiremis (Fig. 7) occurred in rather low densities. Like most other cladocerans it appeared in June and July near the mouth of the Niagara River and at the eastern end of the lake. The species was completely lacking in the northern parts of the lake and in the central part throughout the entire sampling period. Only a few specimens per haul were encountered in the western end during September and October.

Leptodora kindtii (Fig. 8) appeared first and developed earliest in the eastern part of the lake and, in lower densities, near the mouth of the Niagara River. By August it had increased in the eastern part to 3 specimens/cm^2, but was not detected in any of the samples from the western part. The areas of high density characteristically expanded westward along the northern and southern shores. A low abundance in offshore regions was observed during September. Only a few specimens were found in the entire series of hauls from the central part of the lake at that time.

Five other cladocerans were found in much lower densities than the species discussed above. *Chydorus sphaericus* was found from July to September with highest abundance in the eastern and central parts of the lake. *Holopedium gibberum* appeared first in the eastern part in August, but was distributed throughout the lake in September. *Diaphanosoma leuchtenbergianum* appeared only in September, and only in the eastern-most end of the lake. *Polyphemus pediculus* was found only in the eastern part of the lake, during July and August. Finally, a few specimens of *Daphnia galeata mendotae* were encountered in samples near the mouth of the Niagara River collected during September.

COPEPODA

Cyclops bicuspidatus thomasi (Fig. 9), the most abundant copepod in the lake, was more or less evenly distributed throughout nearshore regions of the lake in June except for the area off Toronto, where densities of less than 20 specimens/cm^2 were found. In July, densities were highest in the eastern part, with lower values in the central and western parts of the lake. Low densities occurred along the northern shore in the vicinity of metropolitan Toronto. In August, the population was distributed throughout the lake with maxima of over 400 specimens/cm^2 in the eastern basin and near Toronto. By September densities had decreased in most nearshore areas. Only in the

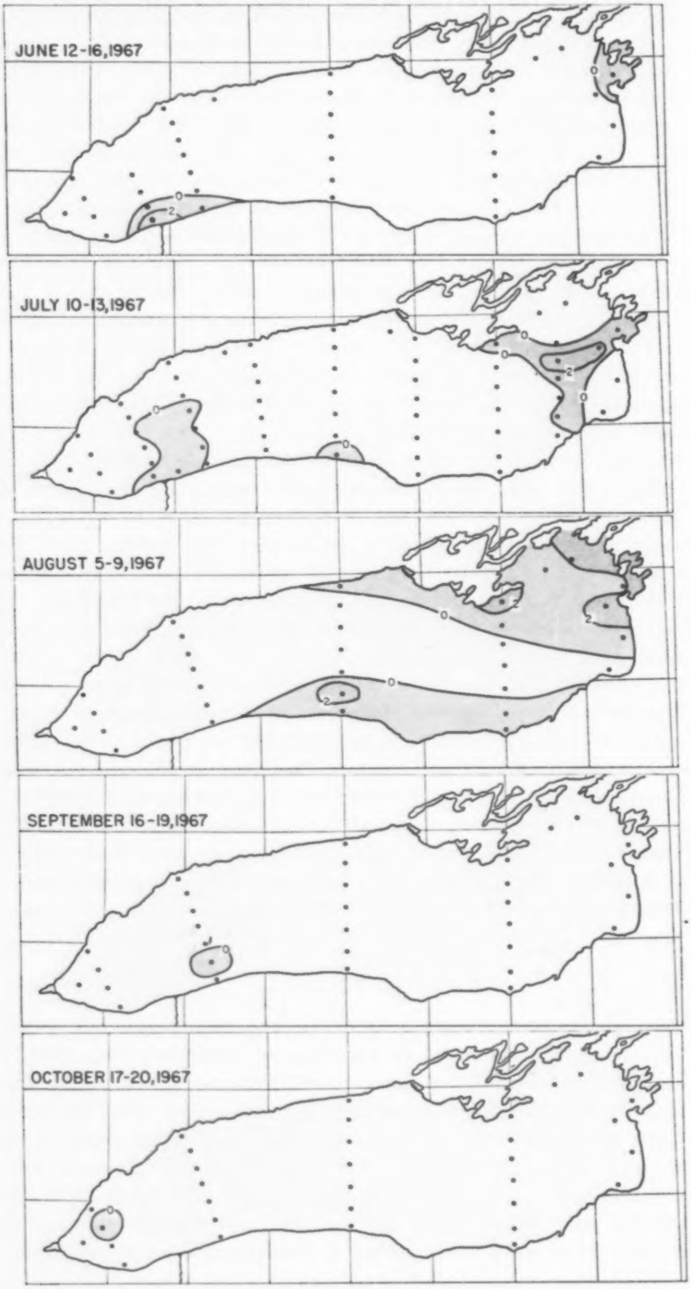
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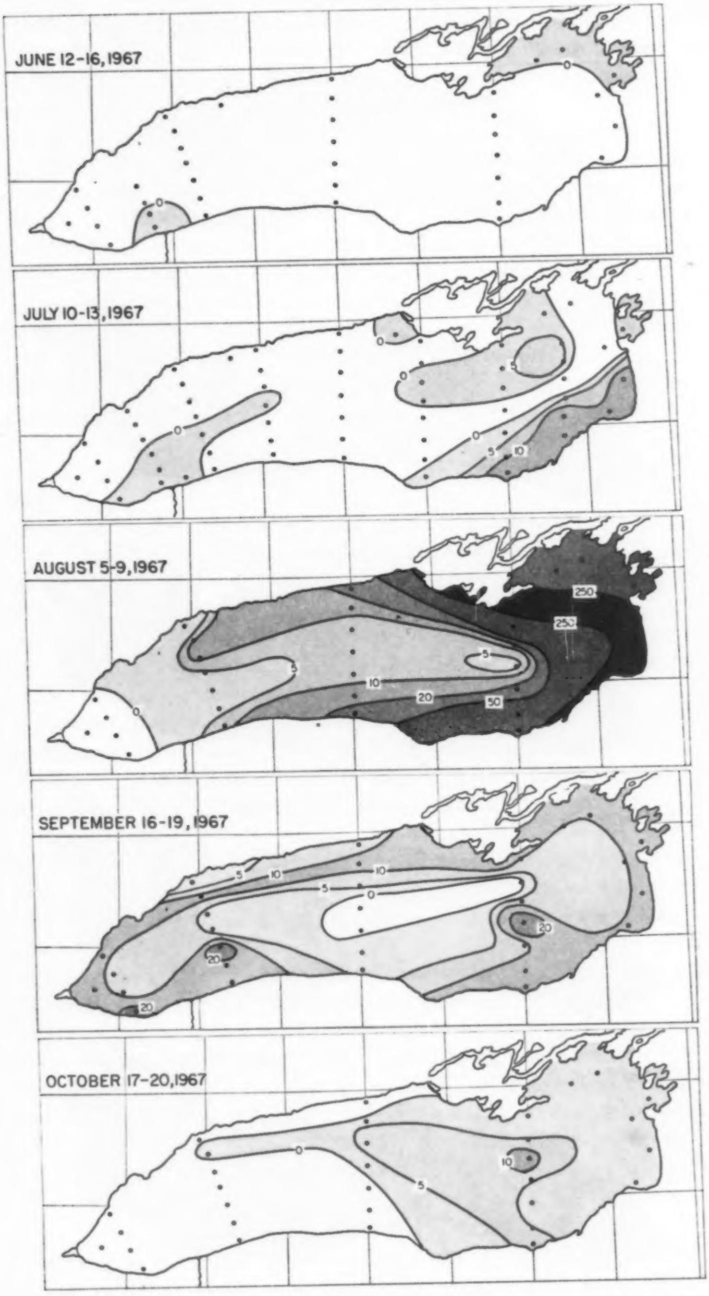
FIG. 7. Distribution of *Daphnia longiremis* from June to October, specimens/cm^3.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JULY 10-13, 1967
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AUGUST 5-9, 1967
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SEPTEMBER 16-19, 1967
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OCTOBER 17-20, 1967
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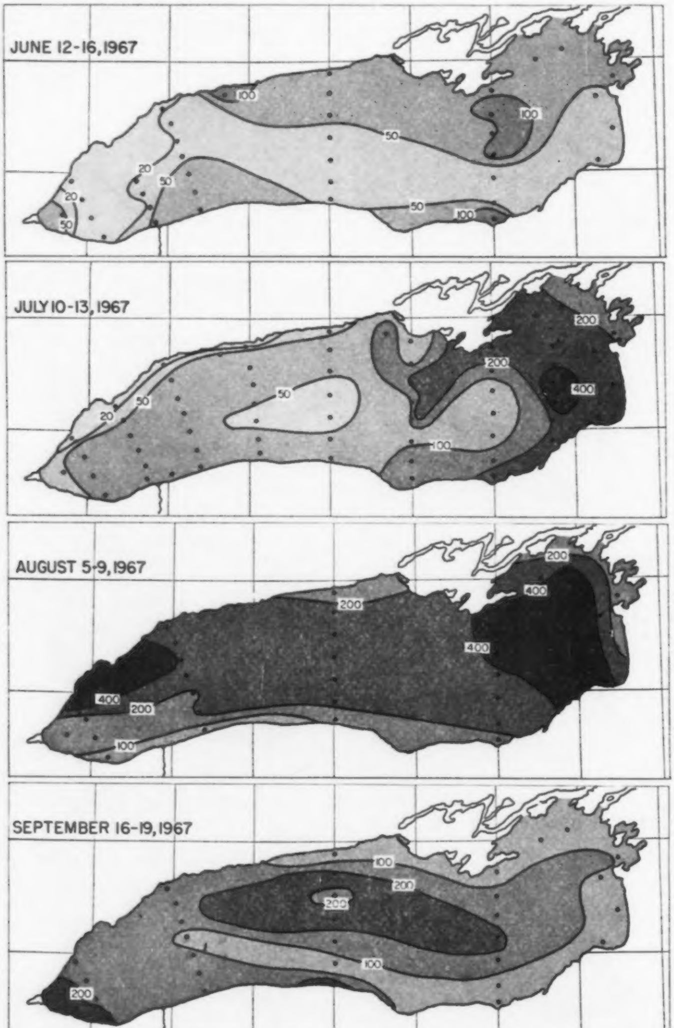
5

FIG. 8. Distribution of *Leptodora kindtii* from June to October, specimens/dcm^2.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AUGUST 5-9, 1967
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OCTOBER 17-20, 1967
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100

FIG. 9. Distribution of *Cyclops bicuspidatus thomasi* from June to October, specimens/cm².

offshore regions did densities exceed 200 specimens/cm². In October *C. bicuspidatus thomasi* was fairly uniformly distributed throughout most of the lake (100–300 specimens/cm²), with lower densities along the northern shore and near the mouth of the Niagara River.

Tropocyclops prasinus mexicanus (Fig. 10) started to develop later than *C. bicuspidatus.* Only a few specimens were encountered in June and July samples, spread over the whole lake. In August, abundance ranged from complete absence in the western part up to a maximum of 50 specimens/cm² in the eastern basin. Densities up to 300 specimens/cm² occurred in different parts of the lake in September with strikingly low values (less than 10 specimens/cm²) near the mouth of the Niagara River. *Tropocyclops prasinus mexicanus* was the most abundant copepod in the eastern part of the lake in September, its density surpassing that of *C. bicuspidatus thomasi.*

Mesocyclops edax and *Cyclops vernalis*, the two other cyclopoids found, were distributed sporadically throughout the lake with densities usually less than 2–4 specimens/cm². The greatest abundance of *C. vernalis* occurred in July in the extreme northeastern end of the lake, with densities up to 27 specimens/cm².

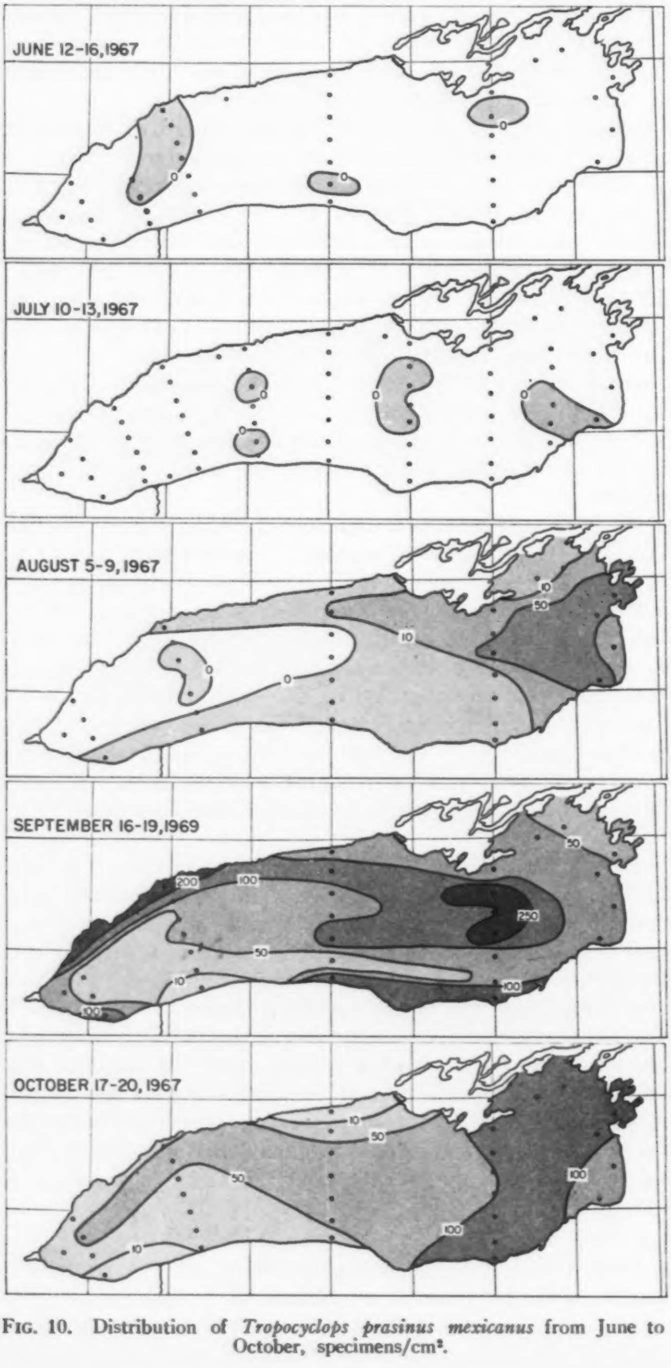
Five species of diaptomids occurred in the samples, all in relatively low abundance (Fig. 11). *Diaptomus minutus, D. oregonensis,* and *D. sicilis* occurred in densities rarely exceeding 10 specimens/cm². *Diaptomus siciloides* was found only in August in one sample from the northeastern end of the lake. A few specimens of *D. pallidus* were observed in October samples from the eastern end. As shown in Fig. 2, *D. sicilis* tended in part to occupy greater depths than those routinely sampled by the vertical hauls, so the abundance may be underestimated by about 50%. Treated as a whole the diaptomids were distributed throughout the lake from June to October, with greatest densities (over 20 specimens/cm²) in the eastern basin. Lowest densities (frequently less than 2 specimens/cm²) occurred near the mouth of the Niagara River.

Eurytemora affinis (Fig. 12) was distributed throughout the lake, though in a rather irregular manner. In June, single young specimens were encountered in individual hauls from the western basin. The highest densities (over 20 specimens/cm²) occurred in July and August in the eastern part of the lake. The region between the Niagara River mouth and Toronto was devoid of *Eurytemora* throughout the entire sampling period.

Limnocalanus macrurus occurred irregularly in samples from all parts of the lake throughout the sampling season. Since the number of specimens was found to be related to the time of sampling it is pointless to mention the results of counts. Occasionally a few specimens of *Mysis relicta* Lovén occurred in samples from all parts of the lakes .The maximum numbers, up to 20 specimens/haul, were in samples taken during the night as would be expected.

DISCUSSION AND CONCLUSIONS

It is evident from the data presented above that there are regularities in the distributional patterns of abundance of crustacean zooplankters in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OCTOBER 17-20,1967
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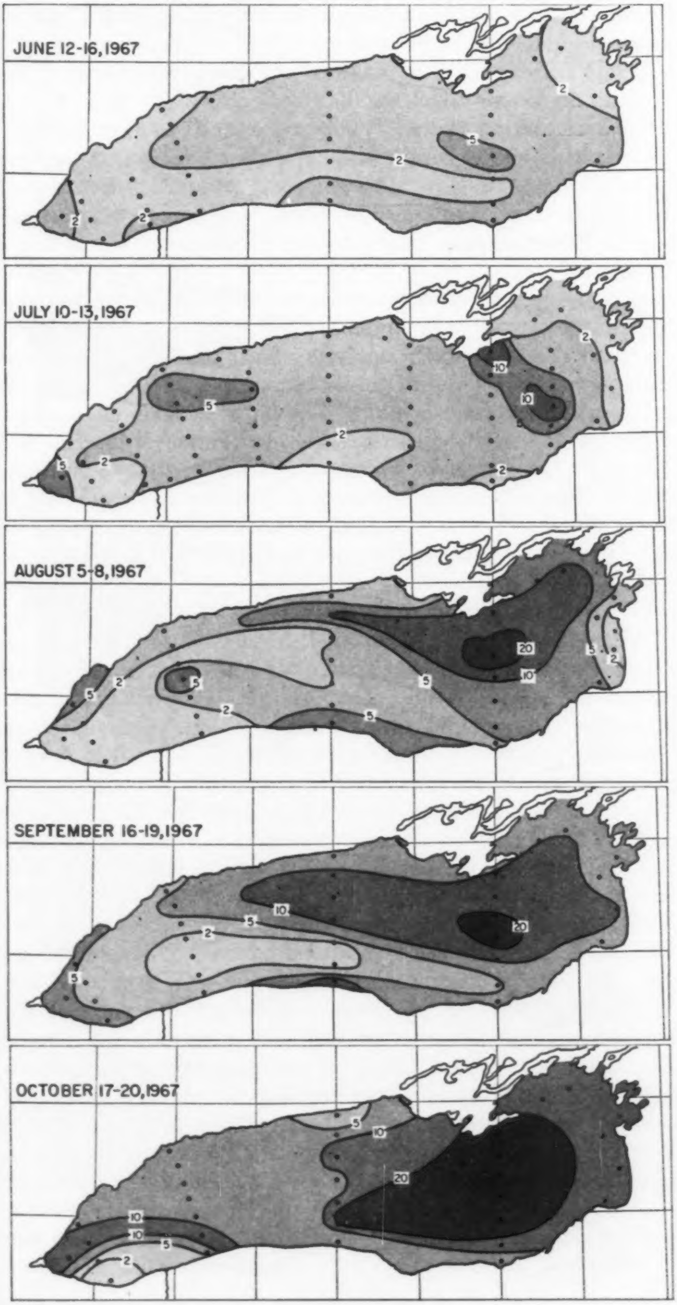
FIG. 10. Distribution of *Tropocyclops prasinus mexicanus* from June to October, specimens/cm².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AUGUST 5-8,1967
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SEPTEMBER 16-19,1967
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OCTOBER 17-20,1967
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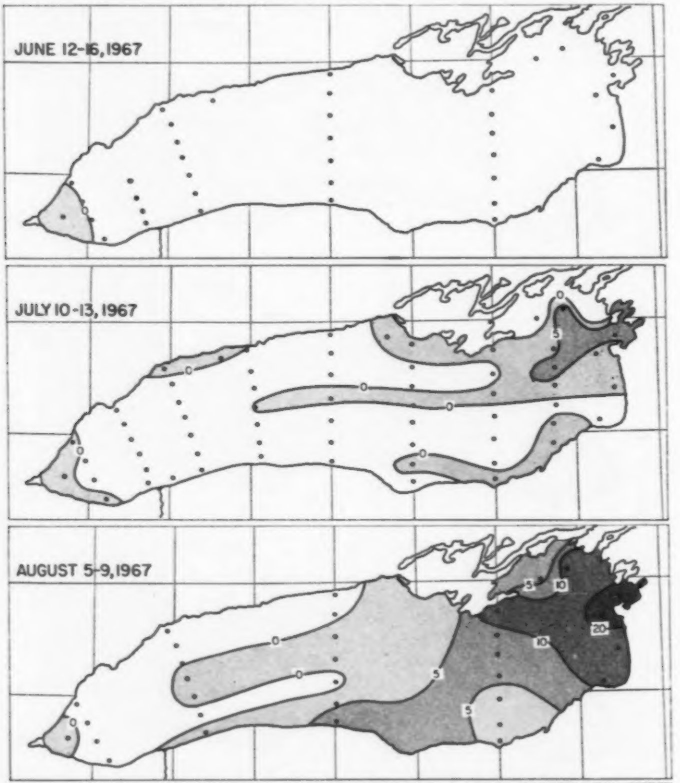
FIG. 11. Distribution of *Diaptomus* spp. from June to October, specimens/cm^2.


JUNE 12-16, 1967
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SEPTEMBER 16-19, 1967
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OCTOBER 17-20, 1967
0
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FIG. 12. Distribution of *Eurytemora affinis* from June to October, specimens/cm².

Lake Ontario. Viewing the data as a whole, most species appeared in June and July in the eastern end of the lake, with zones of abundance later expanding westward. In September, some species such as *B. longirostris* and *C. lacustris* occurred mainly in the western part of the lake, almost disappearing from the east. Others, such as *D. retrocurva* and *C. bicuspidatus thomasi*, were distributed over the entire lake with a tendency toward concentration in deeper offshore regions. By October, the abundance of most species had decreased in the western end with a slight increase in abundance in the eastern end of the lake, and an overall tendency toward a more uniform distribution throughout the lake.

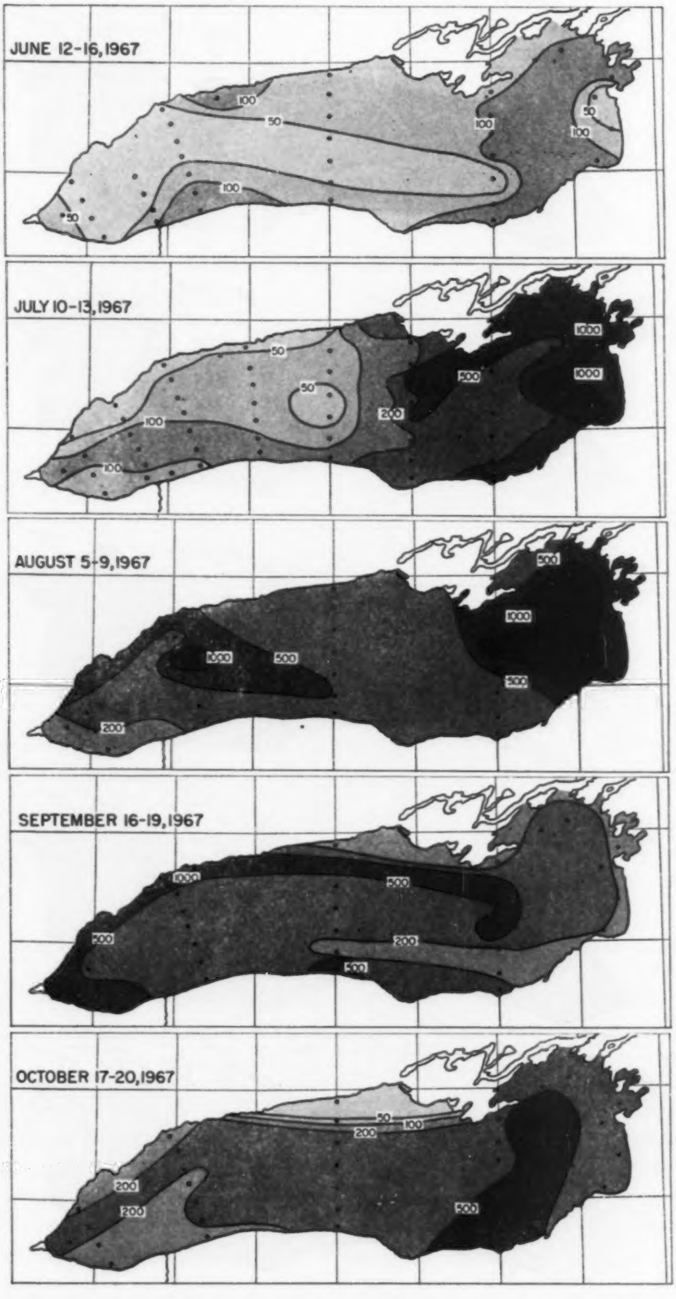
It is important to note that the general westward extension of high abundance zones is contrary to the general direction of wind and water flows, which is from west to east. The major distributional patterns of zooplanktonic abundance, therefore, cannot be explained purely in terms of mechanical transport. The prevailing motions of wind and water do, however, cause important changes in thermal structure, which in turn is positively correlated with the overall pattern of zooplanktonic abundance as shown below. Very simply, the wind piles up warm water in the eastern end of the lake, which permits a greater zooplanktonic abundance there.

The significance of the wind as the ultimate factor controlling the distribution and abundance of zooplankters lies in the effects it produces: increased surface temperatures and an increased depth of the epilimnion toward the east and, to a lesser extent, toward the south. The 10 C isotherm, for example, is twice as deep in the eastern end of the lake in midsummer as it is in the western end, and the average midsummer temperature of the surface water off Oswego is roughly 3 degrees C higher than it is off Toronto. Water temperature and the depth of the epilimnion are both factors that can be expected to exert a controlling influence, directly and indirectly, on zooplankton abundance and development. Temperature acts directly to hasten growth rates and thus production, and indirectly to stimulate the growth of phytoplankton, providing that nutrients and adequate light are available in the environment. The depth of the epilimnion is considered to operate as a capacity factor, increasing the volume of environment available for rapid population growth. To evaluate the relationship between zooplanktonic abundance and the thermal characteristics of the water, the two factors of temperature and epilimnic depth were considered together and expressed in terms of the heat content of a 0–25-m water column, 1 cm^2 in area. The heat content was rather arbitrarily calculated in terms of kilogram calories cm^{-2} using 0 C as the reference point. It would, of course, be more appropriate to use some physiological zero as the reference point, but this demands a more detailed knowledge of the changing patterns of vertical distribution of the species relative to the thermocline than is available. The depth interval of 0–25 m was selected on the grounds that the bulk of the zooplanktonic population occurs there, and also because it afforded the best measure of the epilimnic and metalimnic strata through the summer and autumn seasons.

Figure 14 shows the heat content of the 0–25-m water column for Lake Ontario during the months of observation calculated from bathythermograms supplied by the Canadian Oceanographic Data Centre. The close relationship between the distribution of heat and the overall abundance of zooplankton is obvious: both show gradients increasing toward the east. This is demonstrated for individual species in Fig. 15–19 where abundance from 0–50-m hauls (specimens/cm^2) is plotted against heat content of the 0–25-m layer (kcal/cm^2) for times when each species was at its maximal abundance. The correlation coefficients were highly significant: $r = 0.69$ for *C. bicuspidatus*; 0.80 for *B. longirostris*; 0.77 for *L. kindtii*; and 0.86 for *D. retrocurva*. Such high correlations indicate that temperature and its distribution in the water is the decisive factor governing zooplanktonic abundance during the spring and summer months. Whether this effect is produced directly by an acceleration of zooplanktonic growth rates, or indirectly by increased production of food organisms, has not been ascertained. As the 0–25-m water progressively warmed in the western and central basins of the lake, reaching maximal heat content in September, the relationship between heat content and zooplanktonic abundance became less clear.

The relationship between heat content and average abundance of some of the commoner zooplankters is shown in Fig. 19 for the eastern, central, and western parts of Lake Ontario (see Fig. 14 for the location of dividing lines). For some forms in the central and western areas (*B. longirostris*, *C. bicuspidatus*) the time of maximal abundance preceded the time of maximal heat content, but in most other instances, and for the total zooplankton population as a whole (Fig. 20), the two peaks were co-incident. The same phenomenon is demonstrated in a different fashion in Fig. 21 where the averages of zooplankton abundance in the three parts of the lake are plotted separately by cruise against simultaneous measurements of average heat content. The observed correlation is rather close ($r = 0.80$), particularly when one realizes that the tendency for many zooplankters to show two yearly maxima (Chandler, 1940; Pennak, 1946; Davis, 1954; Patalas, 1954, 1956, 1963) has not been taken into consideration. Actually, a bimodal distribution was observed in the eastern part of Lake Ontario (see Fig. 19, 20), the small second peak occurring in October. In the central and western areas the populations increased more slowly, reaching maximal values during September with no development of a second peak.

The general pattern of the wind over the lake also influences the distribution and abundance of zooplankton on a more localized scale. A case in point is the phenomenon of upwelling that occurs in the vicinity of Toronto as a result of prevailing north westerly winds there (Sweers, MS, 1968). Relatively strong upwelling occurred on July 18, 1967, with surface temperatures along the northwest shore about 8 degrees C lower than along the southern shore (see Fig. 22). The overall abundance of zooplankton at the same time along the northern shore was only one-quarter of that along the southern shore of the lake (see Fig. 13). Similar patterns can be observed in Fig. 3, 6, and 9.


JUNE 12-16, 1967
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JULY 10-13, 1967
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AUGUST 5-9, 1967
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500
200
SEPTEMBER 16-19, 1967
1000
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OCTOBER 17-20, 1967
50
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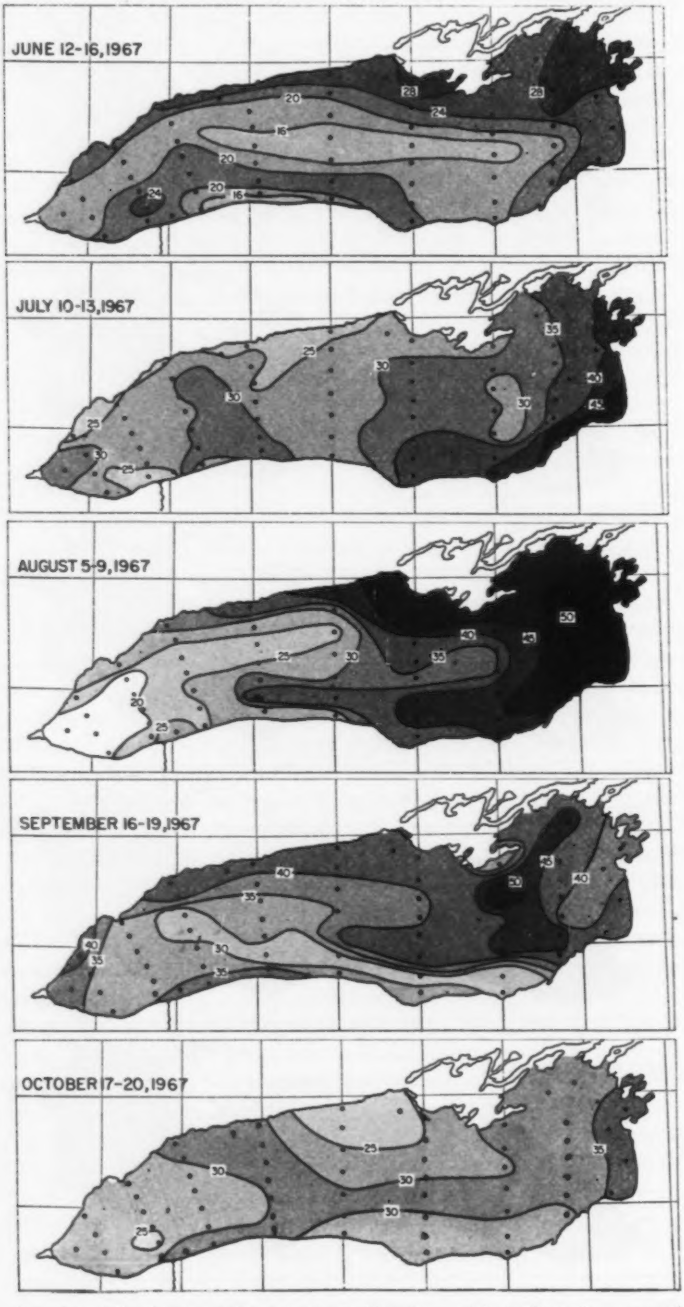
200
200
200
500

FIG. 13. Distribution of total crustaceans from June to October, specimens/cm².


JUNE 12-16,1967
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AUGUST 5-9,1967
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SEPTEMBER 16-19,1967
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FIG. 14. Distribution of heat in the 0–25-m layer of Lake Ontario expressed in kcal/cm².

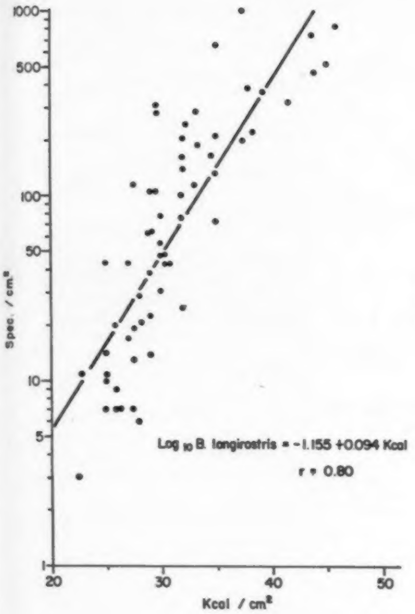


FIG. 15. Relation between the heat content (kcal/cm²) and the abundance (specimens/cm²) of *Bosmina longirostris*, July 10–13, 1967.

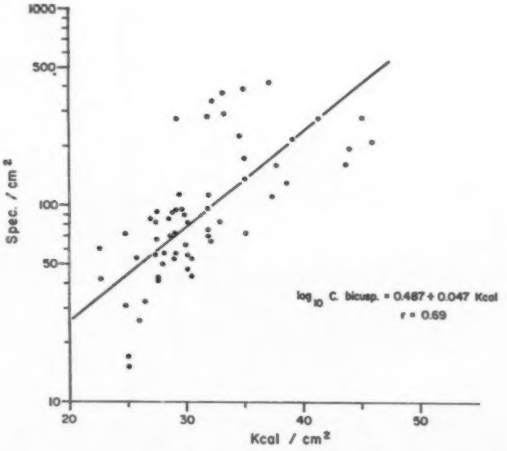


FIG. 16. Relation between the heat content (kcal/cm²) and the abundance (specimens/cm²) of *Cyclops bicuspidatus thomasi*, July 10–13, 1967

The distribution of stations and the time of the sampling do not permit any conclusions regarding the effect of the thermal bar in spring on the distribution of zooplankton. It is possible, however, that the distribution of *C. bicuspidatus thomasi* in June (see Fig. 9) could have been related to the general pattern of heat distribution at that time (Fig. 14).

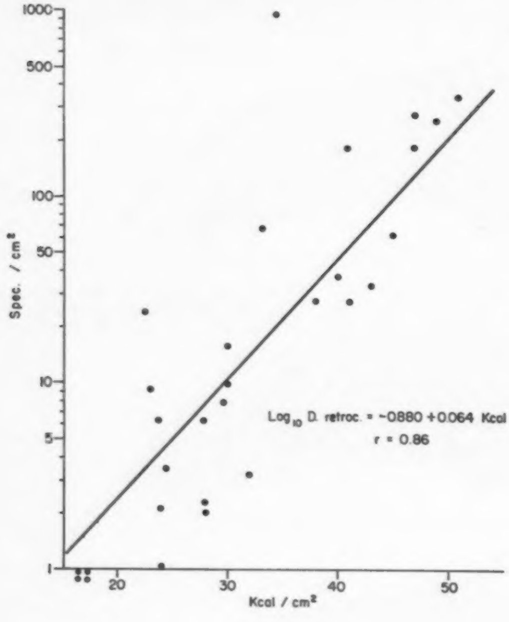


FIG. 17. Relation between the heat content (kcal/cm²) and the abundance (specimens/cm²) of *Daphnia retrocurva*, August 5-8, 1967.

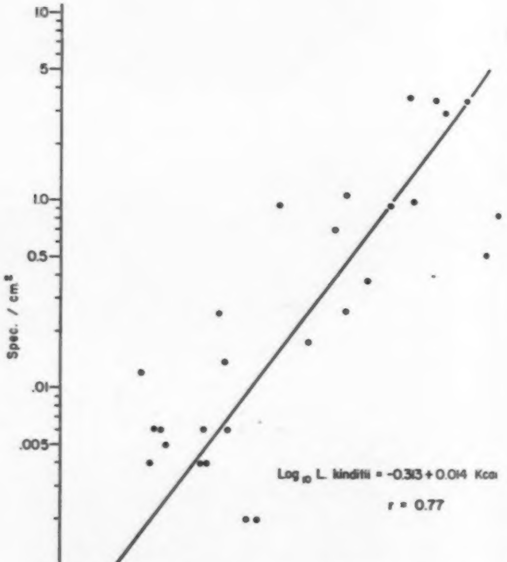


Fig. 18. Relation between the heat content (kcal/cm²) and the abundance (specimens/cm²) of *Leptodora kindtii*, August 5-8, 1967.

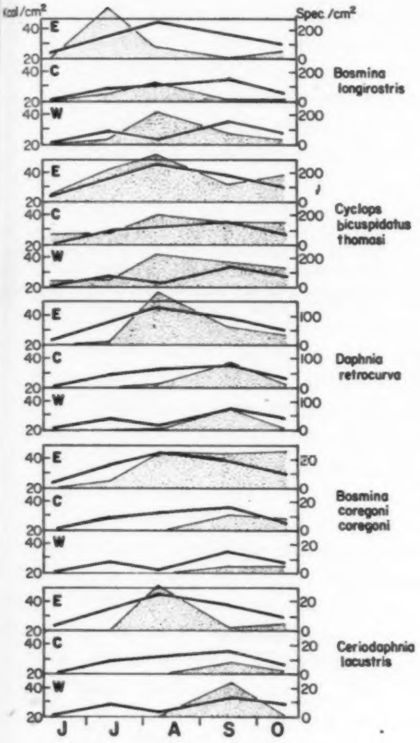


FIG. 19. Seasonal changes in the density of the most abundant species (*stippled area*) and in heat content (*thick line*), both averaged for the eastern (E), central (C), and western (W) parts of Lake Ontario.

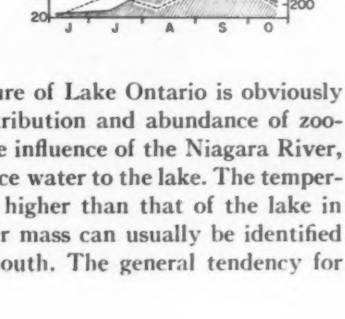


FIG. 20. Seasonal changes in the density of planktonic crustaceans (*hatched area*) and in heat content (*broken line*) averaged for the eastern (E), central (C), and western (W) parts of Lake Ontario.

Although wind-generated thermal structure of Lake Ontario is obviously the primary factor governing the overall distribution and abundance of zooplankton, an additional secondary factor is the influence of the Niagara River, which supplies 80% of the total influx of surface water to the lake. The temperature of the Niagara River input is usually higher than that of the lake in spring and summer, and the flow of the water mass can usually be identified from the pattern of isotherms around the mouth. The general tendency for

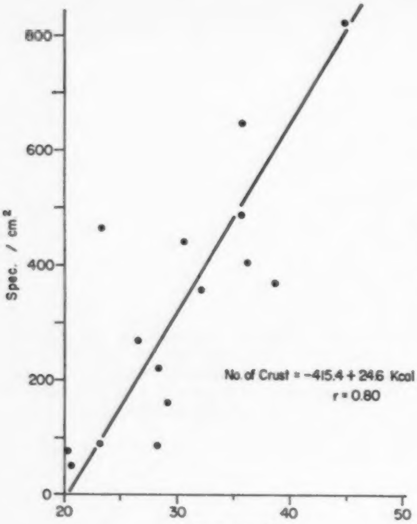
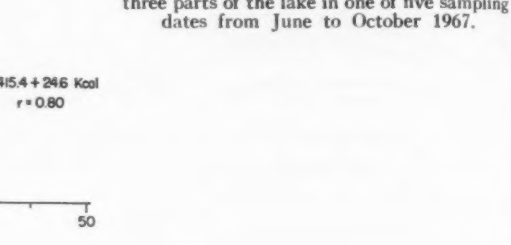


FIG. 21. Heat content and total crustacean planktonic abundance in Lake Ontario. Each point represents an average for one of the three parts of the lake in one of five sampling dates from June to October 1967.

FIG. 22. Surface temperatures in Lake Ontario on July 18, 1967 (based on data from the Lakes Investigation Unit, Meteorological Branch, Department of Transport).



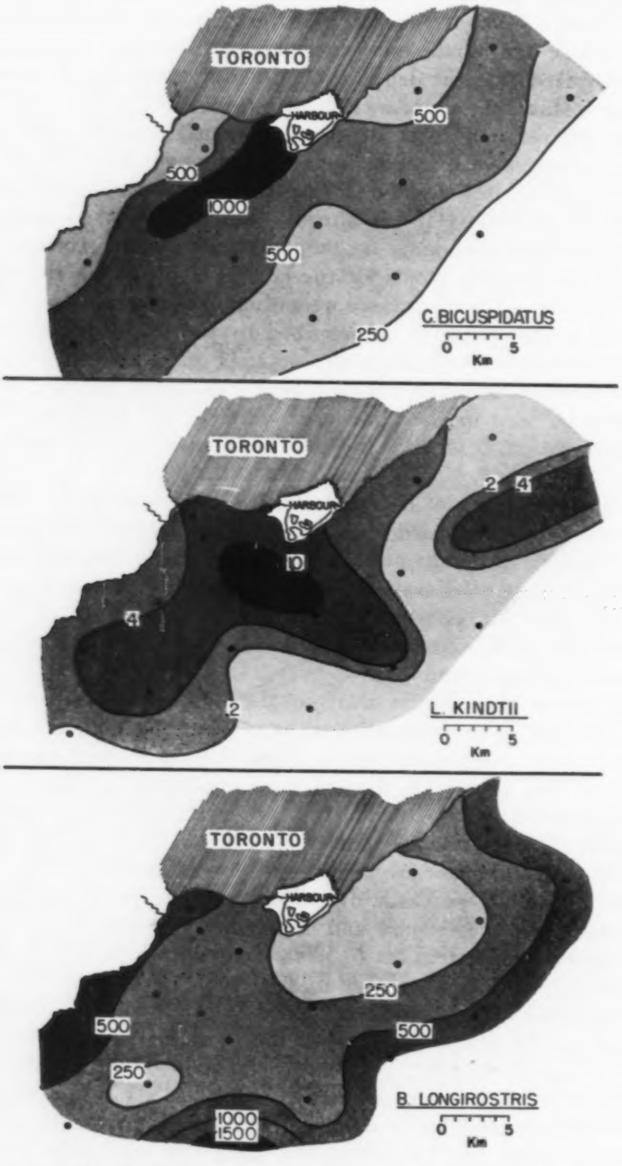
flow is toward the east. Similar patterns were often observed in the distribution and abundance of individual zooplankters: *D. retrocurva* (Fig. 9, July, August); *B. longirostris* (Fig. 3, June, July); *D. longiremis* (Fig. 7, June, July); *Diaptomus* spp. (Fig. 11, June, August, September, October); *C. bicuspidatus* (Fig. 9, June, August, October); *L. kindtii* (Fig. 8, June, July, August); and *T. prasinus mexicanus* (Fig. 10, September, October). Although the details of the patterns may be caused by other factors, there can be little question of the general influence of the Niagara River flow on the overall eastward direction of lines of equal abundance. It is particularly noteworthy that *T. prasinus mexicanus*, a form yet to be reported from Lake Erie, had its lowest abundance in the area adjacent to the mouth of the Niagara River. The general effect of the Niagara inflow on zooplankton was one of enrichment in June and July, and just the opposite in September and October when the river water is cooler than the lake water (see Fig. 14, June vs. October).

The extent to which surface currents modify the distribution of zooplankton in Lake Ontario is not known in detail. This is quite another question from the one discussed above in relation to the thermal structure of the lake in that it involved a physical transport of water rather than changes in the thickness of epilimnic and hypolimnic water masses at different locations. It must also be recognized that the depth distribution of wind-induced current velocities and zooplanktonic abundance do not coincide. Current velocities tend to decrease exponentially with depth in near-surface layers, and often show reversals or changes in direction in the metalimnion and near the bottom. Zooplankters are subject to these motions depending on their vertical position at different times of day. It has already been noted that in most instances the lines of maximal abundance for zooplankters move in a contrary direction to that of the prevailing winds for the lake as a whole. On the other hand, lines of zooplanktonic isoabundance were often found to coincide with generalized surface current patterns as described by Sweers (MS, 1968). There is, for example, a tendency for surface currents to be directed westward in northern areas of the lake and eastward in the southern areas during summer. Comparable patterns can be seen in the abundance patterns for *Diaptomus* in Fig. 11 (August, September), *C. lacustris* in Fig. 5 (September), and *B. coregoni coregoni* in Fig. 7 (September).

This relationship is, of course, inferential and was found only between selected species and generalized summer circulation patterns. Since zooplankton occur at a variety of depths and change their vertical position rhythmically over 24 hr, complex relationships can be expected. A detailed study of the influence of water currents on the horizontal distribution of zooplankton would probably be of considerable interest in relation to the biology of the entire planktonic community.

Local sources of pollution also probably influence the distribution and abundance of zooplankton; however, no permanent effects in any one location were evident from the present study. This may be in part connected with the relatively long generation time of most crustacean plankters relative to the distance over which they could be transported during the same time interval. Nevertheless, there were some indications that waters adjacent to Toronto and Hamilton were temporarily enriched in zooplankton as compared with other areas in the western basin, over and above effects due to heat content. Note, for example, the August and September patterns for *B. longirostris* in Fig. 3, the August pattern for *C. bicuspidatus* in Fig. 9, and the September pattern for *T. prasinus mexicanus* in Fig. 10.

To demonstrate the possible influence of Metropolitan Toronto on the distribution and abundance of zooplankton, a special series of samples was collected on August 9, 1967, from an 8-mile strip of water centered about Toronto Harbour. The results are shown in Fig. 23. It is apparent that the region off Toronto Harbour is significantly different from adjacent offshore and nearshore areas. The location of the centers of maximum density of *C. bicuspidatus thomasi* (over 1000 specimens/cm^2) and *L. kindtii* (over 10 specimens/cm^2) immediately adjacent to the harbour suggests, though does not prove, that these may be related to local sources of pollution. The densities


TORONTO
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L. KINDTII
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TORONTO
HARBOUR
250
500
500
250
1000
1500
B. LONGIROSTRIS
0 5
Km

FIG. 23. Distribution and abundance of *Cyclops bicuspidatus thomasi*, *Leptodora kindtii*, and *Bosmina longirostris* (specimens/cm^2) in Lake Ontario in the vicinity of Toronto on August 9, 1967.

of *B. longirostris*, on the other hand, showed a more or less reverse relationship. Predator–prey interactions could be involved there since *C. bicuspidatus thomasi* and *L. kindtii* are both predatory species. The high concentrations of zooplankton near Metropolitan Toronto were in any case confined to a limited area and could not greatly affect the averages for the western basin as a whole.

The most probable manner in which pollutants could influence the distribution and abundance of zooplankton in Lake Ontario is as follows. Four main pollution centers are situated in the western end of the lake (Toronto, Hamilton, St. Catherines, and the Niagara River complex), and one in the central portion (Rochester). All contribute their pollutional loads throughout the year. Due to effective horizontal mixing of water (Sweers, MS, 1968) the pollutants are probably mixed over the whole surface of the lake, and throughout the volume of the epilimnion during summer. Only when favourable conditions for growth develop (high temperatures and an abundance of food) does the zooplanktonic population respond. Such conditions are created by the prevailing westerly winds earlier in the eastern end than in the western and central parts of the lake. This may also be the reason why localized effects of pollution were observed offshore from Toronto only in August and September, the months with the highest temperatures.

The essence of the above discussion is that the distribution of heat within Lake Ontario, particularly during the spring–summer period, is the major factor governing the overall distribution and abundance of most zooplanktonic species. Upwelling, surface currents, the influx of Niagara River water and pollutants all exert important secondary influences on a more localized scale. The results of this study have important implications to the biology of planktonic communities in the Great Lakes. The full evaluation of the findings is, however, deferred until comparable studies now in progress on four of the other St. Lawrence Great Lakes are completed.

ACKNOWLEDGMENTS

First and foremost I am grateful to Mr Alex Salki for the interest, care, and precision with which he assisted in detailed microscopic analyses. Messrs H. F. Nicholson, Joe Durante, William Burton, and shipboard personnel of the Department of Energy, Mines and Resources of Canada were responsible for the considerable effort put into sample collection. Dr David W. Schindler provided valuable criticism on the manuscript and Mr Hank Sweers was most helpful in making bathythermographic data available to me. Finally I am indebted to Dr J. R. Vallentyne for detailed criticism and suggestions that immensely improved the style and clarity of the manuscript.

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Seasonal Activity and Feeding Pattern of Brown Trout (*Salmo trutta*) in a Dartmoor Stream in Relation to Availability of Food

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CHASTON, I. 1969. Seasonal activity and feeding pattern of brown trout (*Salmo trutta*) in a Dartmoor stream in relation to availability of food. J. Fish. Res. Bd. Canada 26: 2165–2171.

In laboratory tests, brown trout were most active between dusk and dawn from spring to autumn, but the percentage of the total activity that occurred during daylight was higher in the summer than in the other two seasons. Analysis of the variation in the weight of stomach contents during 24 hr in the three seasons showed a higher peak in weight at noon in summer than in the other seasons. The difference was related to an increased consumption of material of terrestrial origin, which other work showed to be most available to the fish between 09.00 and 12.00 hr. No relationship was found between the times of maximum occurrence of benthic and emergent items in the stream and of their consumption by trout. The increase in daytime activity during the summer was also evidently related to increased consumption of terrestrial material.

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INTRODUCTION

ONE GENERAL CONCLUSION that can be drawn from the abundant literature on the feeding habits of brown trout in the European lotic habitat is that the fish consume less benthic food and more material of terrestrial origin in the summer than in other seasons. Although many workers have studied the diet content in relation to the prey (Perkins, 1921; Neill, 1938; Frost, 1945; Nilsson, 1957, 1961; Thomas, 1962; McCormack, 1962), none correlated the activity and feeding behaviour of the trout during different seasons with their known change in diet. The purpose of this present study was to examine this relationship.

METHODS

Fish were taken from the Walla Brook, a tributary of the East Dart in southwest England, by electric stunning and transported to the laboratory in plastic tanks. During the journey the water was aerated with pure oxygen, and upon arrival the fish were immediately placed in the aquarium (1.22 × 0.61 × 0.61 m) in which they were to be studied.

After a 48-hr "settlement period," activity was measured for 24 hr, as in a previous study (Chaston, 1968), by counting the passes that the fish made through three beams of red light. These lights were mounted midway along the outside length of the aquarium and the beams

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were aligned to pass through the water column, parallel to the water's surface, at 5, 24.5, and 41.65 cm from the top of the tank. Three fish were used in each experiment, and three experiments were completed each season in the spring, summer, and autumn of 1967. Activity was expressed as the total number of passes across all beams per hour for the 24-hr period.

The results of the activity experiments were also used to estimate the activity level during the day in all seasons and the positions of the fish in the water column during 24 hr. Daily activity was estimated by examining the percentage of the total activity during 24 hr which occurred between 06.00 and 17.00 hr, an arbitrary period used to represent the hours of daylight. It was necessary, in making the comparison, to use the same time period in all seasons. This period, however, was less than the actual day length that the animals experienced in the summer, and therefore a bias was introduced that slightly underestimated the percentage of activity during the daytime in this season.

The position of the fish in the water column was estimated by calculating the percentage of the total number of passes that were recorded by the upper two light beams during 24 hr.

The daily feeding pattern was studied by examining the stomach contents of three fish taken from the Walla Brook every 3 hr during 24 hr. After the three fish had been obtained, the stunning equipment was moved at least 100 m up- or downstream to eliminate the possibility of the next batch of fish having been disturbed by the previous sampling. Two such programmes were completed in both the spring and summer and one in the autumn.

Immediately upon removal from the stream, the fish were killed and 10% formalin was pipetted into their stomachs. Later they were transported to the laboratory in polythene jars which contained 5% formalin. After identification, the material from the stomachs was dried superficially on filter paper and then weighed. To have a common unit to express all the results, the weights of the stomach contents were calculated as percentages of the weights of the whole fish.

All parts of this study were carried out on 2+ or older fish (age-class data from Horton (MS, 1958)) because the use of only adults excluded the possible effect upon the data of the feeding and activity patterns changing with maturation.

The numbers of samples and activity experiments were small because the number of brown trout in the stream was limited and samples from the population were also required for other experiments (Chaston, 1968, 1969).

RESULTS AND DISCUSSION

In all seasons, activity was greatest between dusk and dawn (Fig. 1) and an analysis of variance indicated that the overall activity pattern did not vary ($P<$

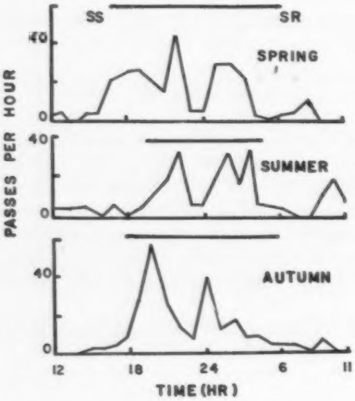

FIG. 1. Total numbers of passes across three light beams per hour in one 24-hr period in each of three seasons (SS = approximate time of sunset, SR = approximate time of sunrise).

0.01) between the three experiments in each season or during the three seasons. However, the animals were more active during the day in the summer than in the other two seasons, the mean percentages of the total activity that occurred between 06.00 and 17.00 hr being spring 14.45%, summer 23.61%, and autumn 9.45%. In addition the fish evidently spent a greater amount of time in the upper part of the water column in the summer, the mean percentages of the total passes that were recorded by the upper two light beams being spring 28.0%, summer 47.68%, and autumn 33.48%.

In the spring and autumn there were peaks in the weight of stomach contents at dawn, midday, and dusk, whereas in the summer there were only two peaks, at dawn and midday (Fig. 2). The absence of an evening peak in the summer may be explained by the fact that the fish had fed more heavily between 09.00 and 12.00 hr than in the spring or autumn.

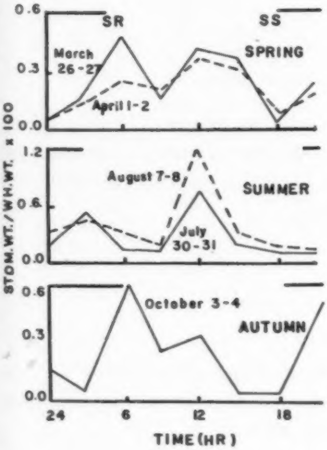

FIG. 2. Weights of stomach contents as percentages of weights of whole fish at 3-hr intervals in 24 hr during three seasons (SS and SR as in Fig. 1).

The mean weights of stomach contents for any time during 24 hr, calculated from the results of the samples taken every 3 hr, were 0.204, 0.285, and 0.252% in the spring, summer, and autumn respectively. This suggests that the fish fed at the same daily intensity during all three seasons, and that the seasonal change in the feeding pattern is an alteration of the times of feeding and not the result of a change in the amount eaten during 24 hr.

To examine the reasons for the peaks in the weight of stomach contents during 24 hr (Fig. 2), the variation in the availability of invertebrate drift (i.e., animals of benthic, emergent, and terrestrial origin found in the water column) in the Walla Brook was compared with the occurrence of these various groups in the stomachs of the trout (Table 1). The data for drift were taken from the results of Elliott's (MS, 1965) 24-hr samples in which the nets of the drift samplers were changed every 3 hr. Thus each of the eight drift samples collected during 24 hr was a record of the animals in the water column during a 3-hr period, and could therefore be directly compared with the stomach analyses as these revealed the diet of the fish for a few hours prior to death.

The number of benthic animals in drift is related to their position (Elliott, MS, 1965) and abundance (Dimond, 1967) on or in the substratum, and these two factors are equally important in deciding the diet of the fish. Therefore it can be assumed that the figures for the occurrence of benthic animals in drift (Table 1) also reflected the daily variation in the availability of benthic fauna to the trout.

The correlation between the percentage occurrences of the three groups of prey in the drift and in the stomachs was estimated with the formula (Moroney, 1962):

$$r = \frac{\sqrt{1/\mathrm{N}(uv - \overline{uv})}}{\sqrt{1/\mathrm{N}(u^2 - (\bar{u})^2)} \cdot \sqrt{1/\mathrm{N}(v^2 - (\bar{v})^2)}}$$

where r = the correlation coefficient, N = the number of samples, v = the % of a material in drift at any one time, and u = the percentage of the material in the stomachs at the same time. The significance of the correlation coefficient was tested by the formula (Moroney, 1962):

$$t = \frac{r\sqrt{\mathrm{N} - 2}}{\sqrt{(1 - (r)^2)}}$$

The correlation coefficients between the percentage occurrences at 3-hr intervals during a 24-hr period of the three groups of animals in the drift and in the stomachs during the three seasons were:

	Spring			Summer			Autumn		
	Benthic	Emergent	Terrestrial	Benthic	Emergent	Terrestrial	Benthic	Emergent	Terrestrial
r	0.430	0.432	0.912	0.389	0.006	0.901	0.499	0.091	No terrestrial material in diet
t	1.38	1.30	5.45	1.13	0.015	5.08	1.41	0.22	
P	>0.20	>0.20	<0.025	>0.20	>0.50	<0.05	>0.20	>0.50	

This analysis showed that the times of maximum availability of terrestrial material in the drift corresponded with the maximum occurrences of this item in the stomachs of the trout ($P < 0.025 > 0.01$), but that no such relationship existed between the feeding of the trout and the maximum occurrence of emergent or benthic material in the drift ($P > 0.20$).

During the summer in the Walla Brook, terrestrial material is the most important component in the diet of 2+ and older brown trout, representing up to 65% of the total diet (Horton, MS, 1958). Also the data (Table 1) on the times of occurrence of the components of drift showed that the maximum

TABLE 1. Occurrences at 3-hr intervals during the day (expressed as percentages of the daily totals) of three types of food in the drift (data from Elliott, MS, 1965) and in the stomachs of the trout for each of three seasons.

Time (*hr*)	Benthic material		Emergent material		Terrestrial material	
	In drift	In stomachs	In drift	In stomachs	In drift	In stomachs
			Spring			
24.00	15	4	12	20	5	9
03.00	7	4	5	0	6	7
06.00	7	6	7	20	7	4
09.00	11	11	5	20	22	22
12.00	12	9	13	20	24	24
15.00	10	24	26	0	15	16
18.00	12	22	18	0	14	10
21.00	26	20	14	20	7	8
			Summer			
24.00	15	11	8	28	11	9
03.00	15	12	8	0	7	4
06.00	6	14	7	0	16	12
09.00	6	13	22	9	15	15
12.00	5	14	13	40	22	25
15.00	3	13	11	15	14	16
18.00	32	12	22	8	7	10
21.00	18	11	9	0	8	9
			Autumn			
24.00	9	12	4	0	No terrestrial material in stomachs	
03.00	12	3	4	0		
06.00	9	22	14	38		
09.00	6	20	17	0		
12.00	6	21	23	0		
15.00	8	4	14	0		
18.00	32	4	14	0		
21.00	18	14	10	62		

amount of terrestrial material occurred in the water column between 09.00 and 12.00 hr. Thus the correspondence between the time of occurrence of maximum terrestrial material and that of maximum consumption suggests that the change in the daily feeding pattern, with a maximum feeding intensity between 09.00 and 12.00 hr, is directly related to the seasonal change in diet.

Terrestrial material was not highly abundant in drift (Table 1) at other times during the 24 hr and therefore it could not be used to explain the peak in the weight of stomach contents (Fig. 2) at dawn in all seasons, or the peak at dusk in the spring and autumn. Elliott (1967) has suggested that the fish in the Walla Brook fed at dawn and dusk when there was increased availability of benthic prey due to the position of the latter on the substratum. It was noted previously that this availability factor would be reflected in the occurrence of benthic material in the drift, and it was therefore possible to test Elliott's hypothesis by examining the occurrence of benthic items in the drift and in the stomachs. The low correlation ($P > 0.20$) between occurrences in the drift and in the stomachs would indicate, however, that the dawn and dusk peaks in the weight of stomach contents (Fig. 2) cannot be explained in terms of the fish feeding during an increased availability of benthic prey. In fact, these two feeding peaks may be explained as the fish feeding at times when the low level of light offered added security against predators such as otters, mink, and the occasional herons that have been observed in the area.

The alteration of the 24-hr feeding pattern in the summer has been correlated with the known change in diet and it was noted that a greater percentage of the trouts' activity occurred during the day, mainly between 09.00 and 11.00 hr (Fig. 1), in the summer than in the other two seasons. In addition the fish spent a larger proportion of time during the summer in the upper part of the water column and this latter conclusion is supported by observations (Chaston, 1969) on the trout in their natural environment. This change in the activity pattern (apparent even after the fish had been moved to the laboratory) is probably also related to the change in diet, because in their natural habitat the fish are feeding more during the day in the summer and consuming terrestrial material that occurs (Norlin, 1967) at or just below the water's surface.

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Open-Water Orientation of White Bass, *Roccus chrysops*, as Determined by Ultrasonic Tracking Methods[1]

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HASLER, ARTHUR D., EDWARD S. GARDELLA, ROSS M. HORRALL, AND H. FRANCIS HENDERSON. 1969. Open-water orientation of white bass, *Roccus chrysops*, as determined by ultrasonic tracking methods. J. Fish. Res. Bd. Canada 26: 2173–2192.

In the spring and summer of 1965, 26 white bass, *Roccus chrysops* (Rafinesque), were displaced to open water on Lake Mendota, Wisconsin, and their paths traced by means of ultrasonic transmitters carried in the stomachs of the fish and receiving gear located on boats. Seventeen fish captured by fyke nets on their spawning ground were displaced 1.6 km west-southwest to a mid lake release point. Two months after the spawning season nine fish, captured by hook-and-line, were displaced 3.1 km east-northeast from their capture area to the same release point. The mean tracking time for all 26 fish was 7.31 hr.

Both the spawning and nonspawning fish maintained steady courses over long distances, and both groups showed a distinct directional preference for the eastern half of the lake where the spawning grounds are located. This open-water orientation helps explain the rapid homing performance, observed in previous experiments, of displaced spawning white bass, and also demonstrates that white bass possess mechanisms for orienting to directional cues present in their natural environment.

Water currents, wind-generated surface waves, and the sun were examined as possible directional cues used by the fish. Although there was some evidence supporting the use of each of these cues, the problem of orientation mechanisms remains obscure. It was determined, however, that the observed movements of the fish cannot be explained as simply passive drift by the water currents.

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INTRODUCTION

THE HOMING ABILITY OF WHITE BASS, *Roccus chrysops* (Rafinesque), has been demonstrated by displacement experiments with marked fish (Hasler et al., 1958; and Horrall, MS, 1961). Fish displaced from their spawning grounds at Maple Bluff and Governor's Island (Fig. 1) to various release points on Lake Mendota returned in large numbers and with good accuracy to the spawning ground where they were originally captured. There was some indication that displaced fish, in addition to homing accurately, returned rapidly

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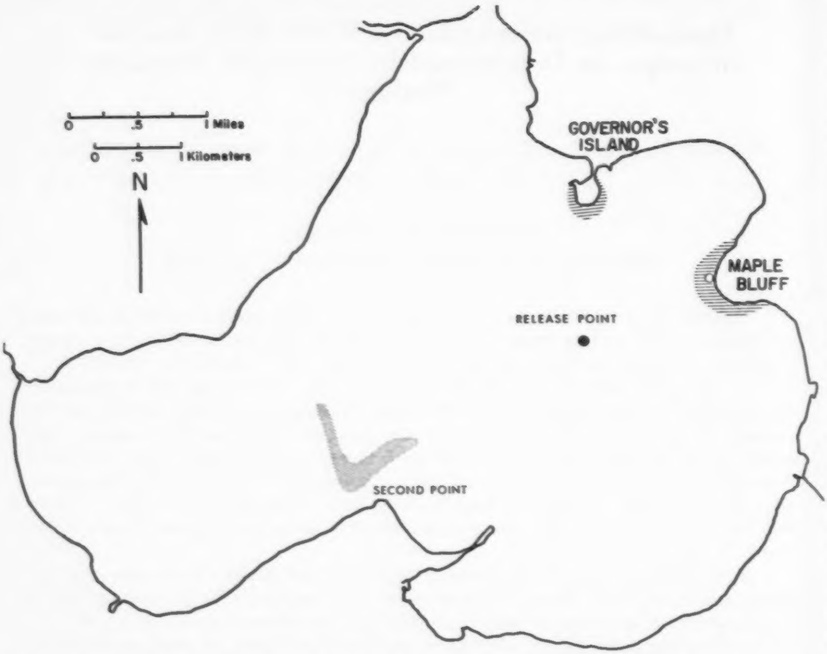

FIG. 1. Lake Mendota, Wisconsin, showing locations of Maple Bluff and Governor's Island spawning areas (*shaded*), the capture sites of the Maple Bluff spawning fish (*open circle*) and the Second Point post-spawning fish (*stippled*), and the release point.

to their spawning ground. This accurate and rapid homing performance suggested an ability to orient toward the spawning ground from open water. In 1957 float-tracking experiments were undertaken to investigate this possibility.

In these experiments (Hasler et al., 1958) a significantly high proportion of displaced white bass, carrying floats attached by monofilament lines, moved in a northerly direction toward the spawning areas on sunny days, but on overcast days or when opaque eye-caps were used the fish usually moved randomly. These field results suggested that white bass possess a sun-compass mechanism for orientation. In these float-tracking experiments tracking time was limited to 1 hr and, therefore, gave only the take-off directions of the fish. In addition, the floats produced drag and restricted the fish to the upper waters of the lake.

After the development of an ultrasonic transmitter for salmon (Trefethen, 1956), we developed a miniature ultrasonic transmitter small enough (a cylinder 9 mm in diameter, 40 mm long, and weighing about 2 g in water) to be carried in the stomach of a white bass, yet powerful enough to transmit a continuous signal (frequency of about 65 kHz) through several hundred meters of water (Henderson et al., 1966). Experiments using plastic tags to simulate the ultrasonic transmitter demonstrated that the per cent recapture (18.8%) at the home spawning ground of displaced white bass with the tag in the stomach was comparable with the per cent recapture (17.5%) of fish without the tag (Henderson et al., 1966). Thus the ultrasonic transmitter does not appear to interfere with homing performance and also allows the fish to be free-swimming, eliminates the problem of drag, and permits tracking times of up to 20 hr.

The objectives of the present study were: (1) to observe the orientation behavior and movement patterns of white bass tracked in open water; and (2) to examine this behavior in relation to possible environmental cues used for orientation. The results are based upon ultrasonic tracks completed during the spring and summer of 1965.

METHODS

In 1965, 21 white bass captured by a fyke net at Maple Bluff (Fig. 1) were tracked during the spawning season between May 29 and June 19. Another nine fish captured by hook-and-line near Second Point were tracked after the spawning season from August 16 through September 3. Except for differences in the location and method of capture, fish in the two groups were treated similarly. The fish were transported from the capture site to a midlake release point marked by a buoy anchored in 22.5 m of water and located 1.6 km from Maple Bluff, 1.5 km from Governor's Island, and about 3.1 km from the capture area near Second Point. After an ultrasonic transmitter was inserted into the fish's stomach, the fish was released at about 1000 hr (CST) and tracked until late in the afternoon from boats equipped with special receiving apparatus (Henderson et al., 1966).

SELECTING AND PREPARING THE FISH

The large numbers of fish captured in fyke nets during the spawning season permitted selection of proven homers, i.e. fish that had been caught in previous spawning seasons at Maple Bluff and had been marked by a characteristic fin punch. Only male fish were used because males remain on the spawning ground much longer than females (Horrall, MS, 1961) and, hence, would presumably have a greater motivation to return there after displacement. Summer fish from the Second Point area included both males and females because external determination of sex is inexact in white bass after the spawning season.

Each fish together with at least one spare fish was transported by boat to the release site (about 5–10 min travel time) in an open aluminum container 40 cm in diameter, 40 cm deep, and filled to a depth of about 25 cm with lake water. Prior to inserting the ultrasonic transmitter, a scale sample was taken, the fish's total length measured, and the soft dorsal fin punched for identification in case of recapture. The transmitter was inserted into the stomach via the mouth by means of a syringe made of thin-walled plastic tubing. The fish was then observed in the container for 8–32 min (mean of 14 min) for possible injury or for regurgitation of the transmitter. A multi-hoop release net (1 m in diameter, and 1.5 m high) suspended from the release-point buoy was used to hold the fish for an additional period of 7–176 min (mean of 96 min) to assure recovery from the handling and tagging procedures. To initiate tracking, the bottom of the net was opened by a special release mechanism, allowing the fish to swim out without additional handling.

TRACKING TECHNIQUES

Tracking was conducted from two barges simultaneously or from a single barge. When two vessels were used the position of the fish relative to the boats was determined by triangulation, which permitted position determinations to be made without moving close to the fish. Location on the lake was determined by sextant sightings using landmarks on shore. Readings were made at a mean time interval of 19.2 min. Because considerable errors resulted from the large number of simultaneous angle measurements required by this technique, it was abandoned after tracking six fish (no. 4, 6, 7, 8, 10, and 12).

The remainder of the fish were tracked from a single vessel. At a mean time interval of 26.6 min the boat was maneuvered close to the fish and sextant readings were taken to determine location. A position close to the fish could be judged by signal strength and loss of signal directionality. Between position determinations the tracking vessel was allowed to drift away from the fish but remained within range of the signal.

The positions derived from the sextant readings were plotted on a map of Lake Mendota using a three-arm protractor. Successive points were joined by straight lines. The points (black circles) shown in Fig. 6 through 10 do not, however, represent these positions but are time markers showing the approximate locations of the fish at 1-hr intervals. There were usually two or three position determinations between successive time markers.

The ultrasonic transmitter used in 1965 did not provide information on the swimming depth of the fish. In subsequent years we have found the transmitter to be sensitive to large temperature changes and now know that tracked white bass generally do not penetrate the thermocline (usually begins at about 10 m depth).

ENVIRONMENTAL DATA

Several environmental factors which could serve as cues or influence the orientation of fish were measured during every track. Cloud cover, sun visibility, wave height, and rain conditions were all noted at the time of each position reading. Water currents, light penetration, and a temperature profile were measured two or three times during a track from a small boat anchored near the location of the fish. The sites of these measurements were often as much as 300 m from the fish's location, but in the pelagic regions of the lake these factors were assumed not to vary appreciably over this distance. The speed and direction of water currents were measured with drogues (modified from Shulman and Bryson (1961) to measure 46 cm on a side) set at 1 m, 7 m, and within the thermocline at about 12 m. A rangefinder and hand-held compass were used to measure the distance and direction traveled by the drogues over a known time interval.

Continuous records of radiant energy (sunlight) in air were obtained from the Truax Weather Bureau (7 km northeast of the release point) during the spawning period, and from the University of Wisconsin Solar Energy Laboratory (4 km south of the release point) during the post-spawning period. The former record was made from an Eppley pyrheliometer and the latter from a silicon photocell. Because these records clearly showed the effects of clouds passing over the sun, they provided information concerning the visibility of the sun at all times during each track. To extract this information from the charts, criteria were established to determine the position of the recording stylus at the time the sun became invisible.

Records of wind speed and direction were obtained from the Truax Weather Bureau.

EVALUATION OF METHODS

Though an attempt was made to standardize the selection and handling procedures of the tracked fish, in practice consistency was hard to maintain under field conditions. We examined the track to see if the differences in directional characteristics might be associated with age differences of the fish (ages listed in Table 1). No apparent association was observed. While tracking salmon, Johnson (1960) found that the longer the fish were held in a live box the sooner they moved out of the release area. To test this for white bass, the time required for each fish to move 200 m from the release point was compared with: (1) the total time the fish was in captivity[3]; (2) the time the fish was held out of the lake in a container; and (3) the time spent in the release net after inserting the transmitter. No statistically significant correlation was found for any of these comparisons.

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[3] For the spawning-season tracks this does not include the time spent by the fish in the fyke net prior to the fish's removal.

The single vessel technique raised the possibility of disturbing the fish at the time of each position determination. Because of this possibility the direction of approach to the fish was varied to avoid biasing the fish's orientation. Fish tracked by this method maintained steady courses for several position determinations, and their choice of direction was not significantly different from that of fish tracked by the triangulation method, where the vessels stayed further from the fish. Moreover, pleasure boats passing near had no observable affect on the fish's movements. Johnson (1960), while tracking salmon with ultrasonic transmitters on the Columbia River, found that attempts to disturb these fish with a motor boat failed to cause them to change their course or rate of travel.

RESULTS AND DISCUSSION

Four out of the 21 fish released during the spawning season were recaptured in the Maple Bluff fyke net during the same spawning period: fish 2, 4, 14, and one other not numbered here because it was lost shortly after being released. This 19% recapture value compares favorably with the 18.8% value found by Henderson et al. (1966) mentioned previously.

A total of eight fish (Table 2, column 1) had reached the littoral zone of the lake by the end of the track. Of the six spawning-season fish reaching this zone, three (no. 4, 5, and 7) arrived at Maple Bluff during the track.

Four out of the 30 fish released for tracking are excluded from this report because three were lost shortly after their release and one was part of another experiment. The mean tracking time for the remaining 26 fish was 7.31 hr with a range of 4.13–10.62 hr (Table 1, column 6). In Fig. 6 through 10, the tracks of all 26 fish are ordered chronologically.

SPAWNING VS. POST-SPAWNING TRACKS

The distributions of spawning and post-spawning tracks were remarkably similar (Fig. 2 and 3, respectively). The movements of fish in both series were confined to the eastern half of the lake, and the empirical mean vectors (Batschelet, 1965) for both distributions after 5 hr of tracking were toward Maple Bluff (Fig. 4). This similarity is noteworthy considering the differences in the time of year and in the locations and methods of capture of the fish.

The similarity between the angular distributions of fish tracked during and after the spawning season was tested statistically by using the rank-sum and run tests described by Batschelet (1965). These nonparametric tests evaluate the degree of intermixing between the angular distributions of points from two samples. These analyses were performed with the 1- and 5-hr distributions (Fig. 4), and indicated no difference between the two series at $P = 0.05$.

Figure 4 gives the angular position of each fish in relation to the release point at hourly intervals, but does not take distances or speed of movement into account. These other factors, as well as changes in angular position, were included by calculating the centers of mass (Batschelet, 1965) from the positions of the fish at 1-hr intervals after release (Fig. 5). It is evident that the centers of mass for both the spawning and the post-spawning series moved away from the release point toward Maple Bluff at approximately the same rate.

TABLE 1. Information on individual white bass tracked during the spawning and post-spawning seasons in Lake Mendota, 1965.

Fish no.	1 Date	2 Total length (*mm*)	3 Age in years	4 Previous years captured at Maple Bluff	5 Release time (*CST*)	6 Tracking time (*hr*)	7 Swimming distance (*km*)	8 Mean observed swimming speed (*cm/sec*)	9 Mean speed of current at 1 m (*cm/sec*)	10 Maximum[a] mean error in direction
					Spawning					
1	May 29	293	4	–	1018	5.35	1.86	9.7	7.6	52°
2	30	379	7	1964	0921	10.62	5.07	13.3	4.7	21
3	31	315	5	–	1057	8.63	3.02	9.7	8.4	60
4	June 1	321	5	1964, 1965	0952	6.13	3.39	15.4	4.9	18
5	2	317	4	–	1409	5.88	4.51	21.3	4.3	12
6	3	306	4	–	0956	5.22	3.33	17.8	5.6	18
7	4	315	4	1964	0922	8.88	5.08	15.9	6.1	23
8	7	293	4	"	0922	7.15	3.31	12.9	5.7	26
9	8	304	4	"	0951	8.18	4.61	15.7	8.2	32
10	9	297	4	"	0851	9.00	5.15	15.9	6.7	25
11	10	300	3	"	0924	8.88	3.95	12.4	6.7	33
12	11	306	4	"	0827	7.47	3.81	14.2	5.1	21
13	14	284	3	"	1011	9.12	3.75	11.5	4.8	25

14	15	329	5	1965	0950	5.75	2.30	11.1	5.0	27
15	16	293	3	1964	0917	4.13	1.84	12.4	8.8	45
16	18	301	4	1964	0945	6.87	1.18	4.8	6.5	180
17	19	278	3	1964, 1965	0929	7.63	2.73	10.0	13.3	180
Mean	–	307.7	4.1	–	0954.2	7.35	3.46	13.2	6.6	29.2[b]
					Post-spawning					
18	Aug. 16	264	2	–	1023	4.95	1.66	9.4	4.5	29
19	18	280	3	–	0933	5.73	2.42	11.8	4.7	23
20	19	309	–	–	0917	8.03	4.83	16.7	4.7	16
21	20	285	3	–	0943	8.15	6.05	20.6	6.8	19
22	24	308	4	–	0930	7.55	2.74	10.1	4.7	28
23	26	273	3	–	0952	7.65	3.30	12.0	7.8	41
24	31	297	4	–	0945	7.18	1.87	7.2	8.7	180
25	Sept. 2	292	4	–	0949	8.08	3.37	11.6	6.9	36
26	3	279	3	–	0937	7.93	1.60	5.6	3.0	32
Mean	–	287.4	3.2	–	0943.2	7.25	3.09	11.7	5.8	28.0[b]
Mean, all fish	–	300.7	3.8	–	0950.4	7.31	3.33	12.7	6.3	28.8[b]

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[a] The error is calculated by taking the arcsin of the ratio of mean current speed at 1 m to mean swimming speed. Where this ratio is greater than one, the error will always be 180°.

[b] The tracks where the error was 180° were not included in this calculation.

TABLE 2. General observations on five factors for individual white bass tracked during the spawning and post-spawning seasons in Lake Mendota, 1965.

	1	2 Swimming direction similar to	3	4	5
Fish no.	Fish reached littoral zone	Direction of current at 1 m	Wind direction	Surface flat calm for at least part of track	Sun invisible for at least part of track
		Spawning			
1		×	×		×
2	×	×		×	
3					×
4	×			×	×
5	×				×
6		×	×		×
7	×				
8					
9	×			×	×
10		×	×	×	
11		×		×	
12	×			×	
13					
14					
15					
16				×	
17		×	×		
		Post-spawning			
18			×		×
19					
20				×	
21		×		×	×
22	×	×	×		×
23	×	×		×	
24		×	×		
25		×	×		
26			×		×

EVIDENCE FOR NONRANDOM ORIENTATION

The nonrandom nature of the open-water movements of the fish is shown both by the angular distributions of composite tracks and by the directional characteristics of the individual tracks.

For both spawning and post-spawning fish the majority of tracks were concentrated in the northeast quadrant (north to east) relative to the release point with very little movement west of this point (Fig. 2, 3). The angular distributions given in Fig. 4 provide a more quantitative means of presenting the data. Owing to the similarity between the spawning and post-spawning series both groups were treated as samples from the same statistical

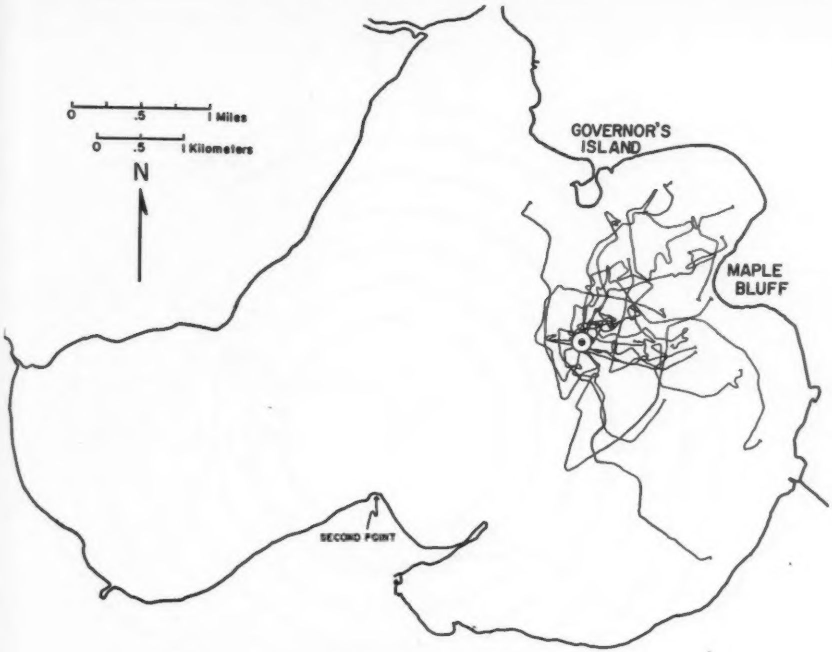


FIG. 2. Composite diagram of 17 tracks of spawning fish.

population. The 1- and 5-hr distributions in Fig. 4 were tested statistically for randomness against the null hypothesis of a uniform circular distribution — here uniformity was taken to imply randomness. The test used was a modification of Smirnov's test (Batschelet, 1965) and showed that the distribution at 1 hr was random but that at 5 hr was nonrandom. This change from randomness to nonrandomness indicates that those fish initially moving westerly later changed direction.

In many tracks the white bass maintained a steady course for long periods — good examples are tracks 6 and 21. One cannot say exactly how steady these courses really were because the paths between position determinations are not known. However, if several successive determinations lie roughly on a straight line one can infer that the direction of the path between these points was quite steady. The ability to maintain steady courses and to move into a specific sector of the lake is not what is expected from random orientation. This suggests that white bass possess mechanisms for orienting to directional cues present in their natural environment.

Environmental Factors and Orientation

It is possible the observed paths of the fish were due entirely to passive drift by the water currents. Since the swimming depth of the fish was not

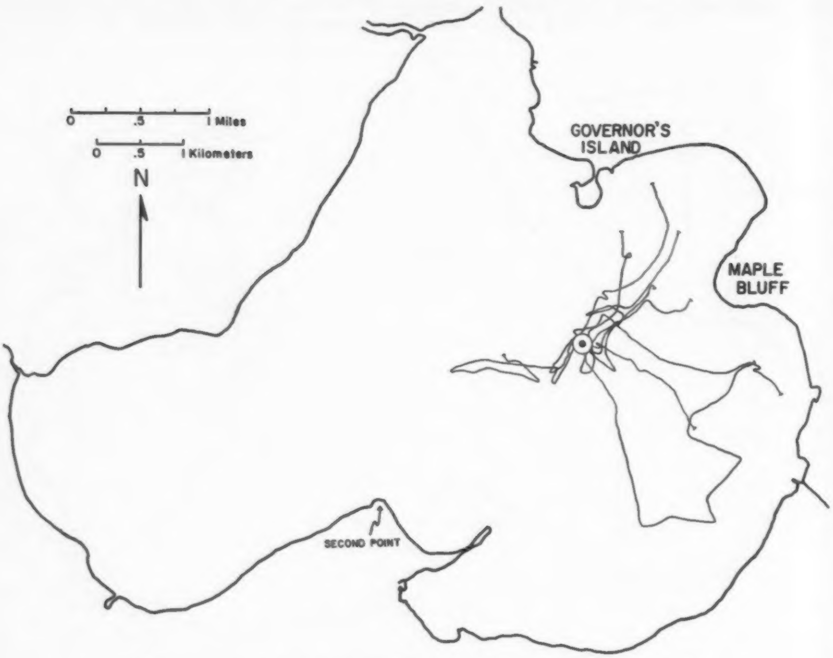


FIG. 3. Composite diagram of nine tracks of post-spawning fish.

known such a possibility cannot be examined directly. A few general observations suggest, however, that passive drift probably occurred infrequently. The mean observed speed of the fish (unweighted mean 12.7 cm/sec, *n* = 26, range 4.8–21.3 cm/sec) was considerably greater than that of the current at 1 m (unweighted mean 6.3 cm/sec, *n* = 26, range 3.0–13.3 cm/sec), which was usually the fastest current measured during any one measurement series. Comparisons between swimming and current speeds within individual tracks indicate there were large segments of each track where observed swimming speed exceeded even the fastest currents present for that particular period. This can be seen in the figures by comparing the distance traveled by the current at 1 m in 1 hr (see Fig. 6 for explanation) with the distance traveled by the fish in 1 hr. There were five tracks (no. 1, 16, 17, 24, and 26) where the paths may have resulted from passive drift because swimming and current speeds were similar throughout the entire track. The similarity in mean swimming speed and current speed at 1 m for fish 3 (Table 1, column 8) should not be interpreted as indicative of continual passive drift because there were several segments of the tracking where observed swimming speeds far exceeded the fastest current speeds. It appears, then, that passive drift occurred infrequently, and that active swimming was involved in most of the tracks.

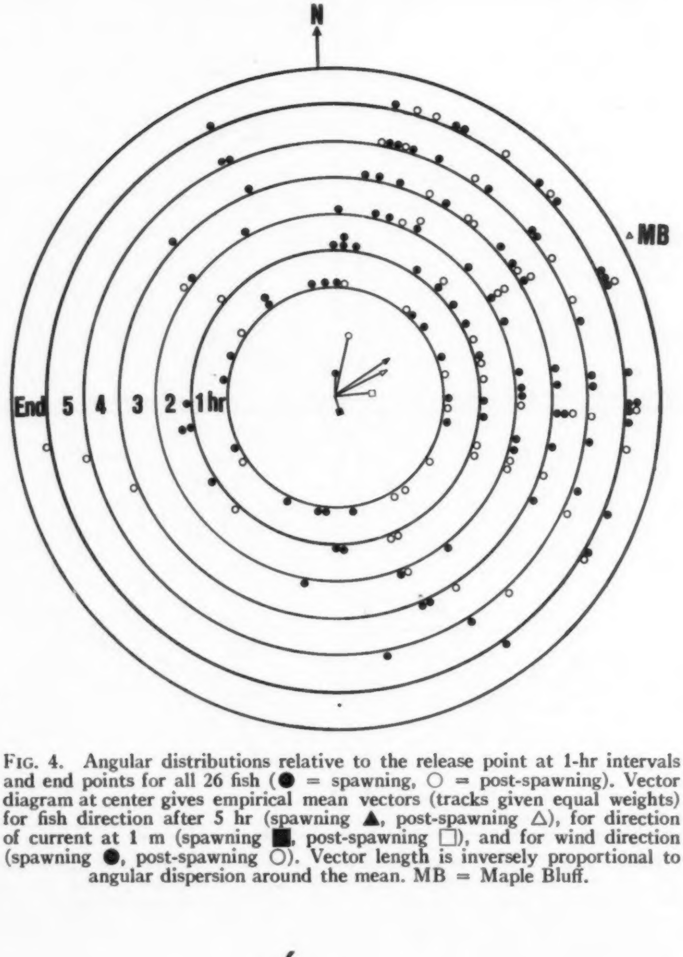


FIG. 4. Angular distributions relative to the release point at 1-hr intervals and end points for all 26 fish (● = spawning, ○ = post-spawning). Vector diagram at center gives empirical mean vectors (tracks given equal weights) for fish direction after 5 hr (spawning ▲, post-spawning △), for direction of current at 1 m (spawning ■, post-spawning □), and for wind direction (spawning ●, post-spawning ○). Vector length is inversely proportional to angular dispersion around the mean. MB = Maple Bluff.

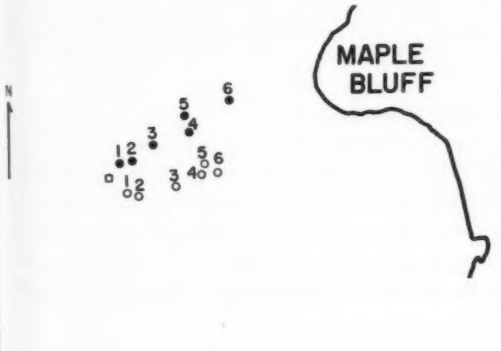


FIG. 5. Centers of masses for distribution of spawning (●) and post-spawning (○) fish at hourly intervals after release (1–6 hr).

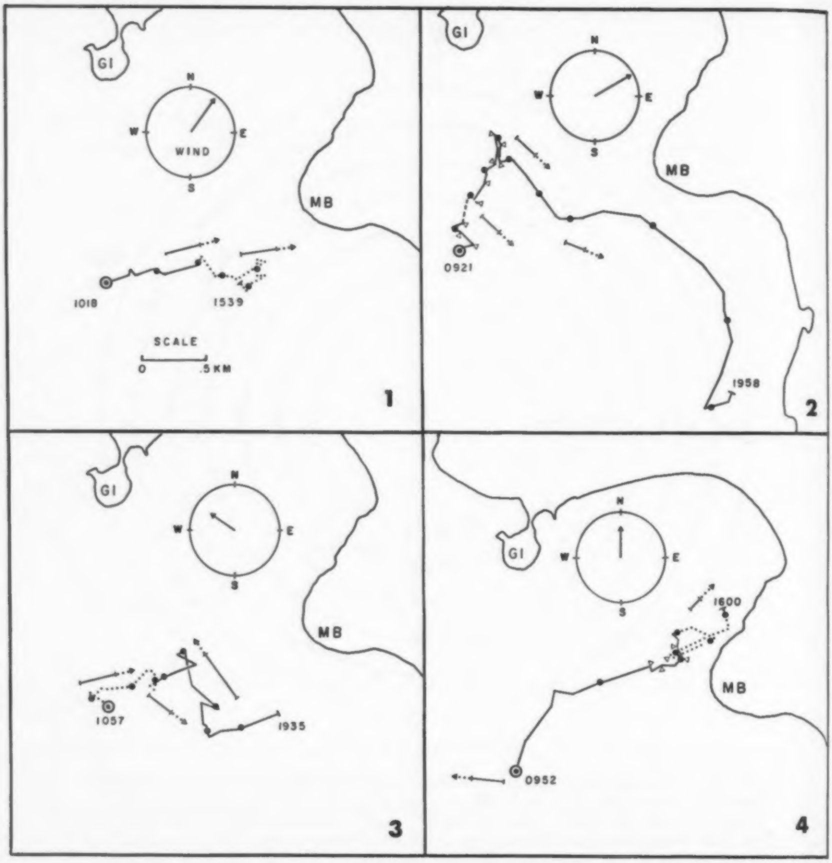
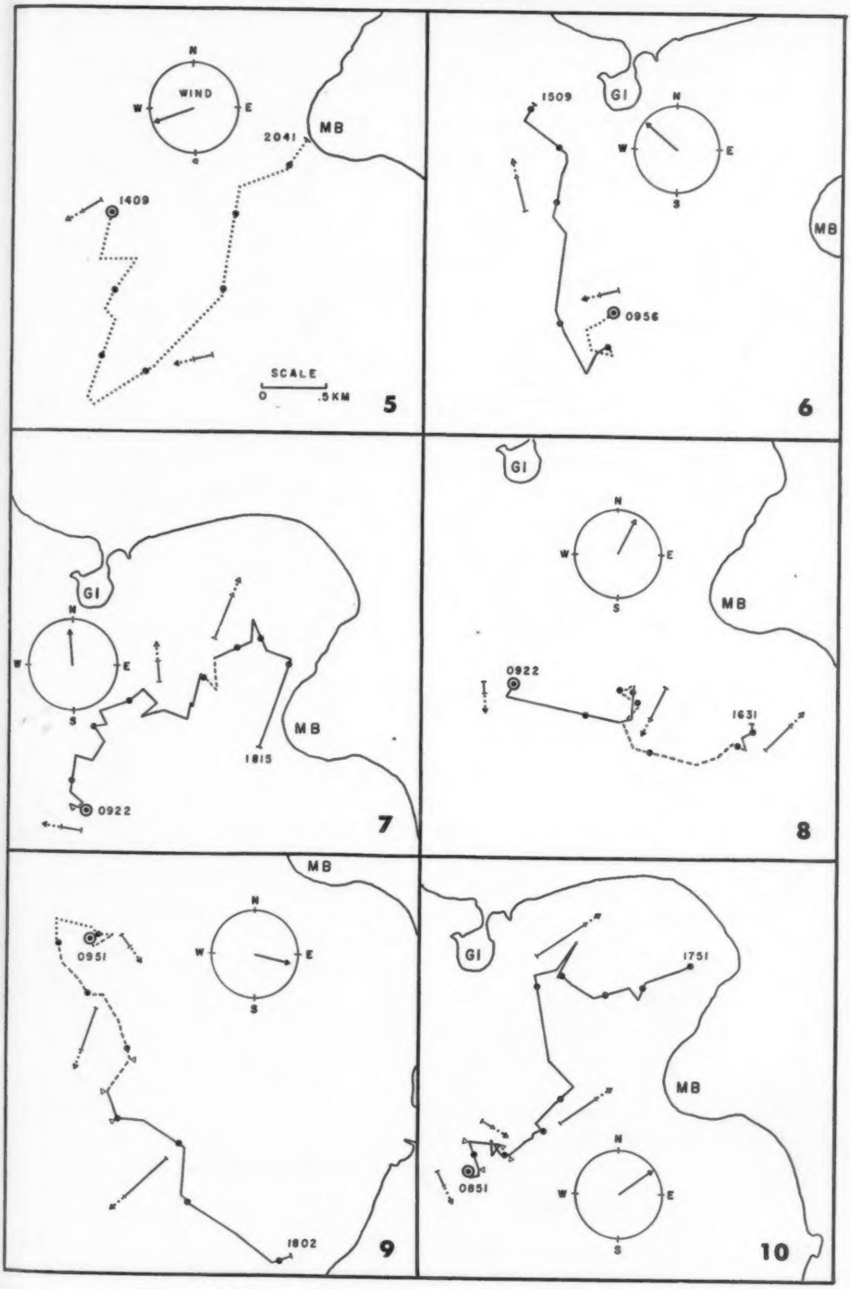


FIG. 6. Tracks of fish 1–4. The vector within the circle is the empirical mean wind vector, the length of which is inversely proportional to the angular dispersion around the mean. The vectors near the fish's path indicate the direction of the current at 1 m (*dotted line*) and the distance it travels in 1 hr (*solid line*). The bases of these vectors are positioned to indicate time of measurements (not location). Solid circles (●) along the track are placed at 1-hr intervals starting from the time of release, and the open triangles (△) indicate that surface waves were absent. The lines joining the position determinations are coded for sun condition: —— sun continuously visible; – – – – sun intermittently visible; and .... sun continuously invisible.

The observed path of the fish is necessarily the vectorial resultant of the movement of the fish relative to the water and the movement of the water itself. Ideally one would like to make corrections for these drift components so that the fish's true orientation and swimming speed can be examined. Such corrections cannot be made here because swimming depth was not known. The directional errors caused by using the observed (Fig. 6 through 10) rather than the corrected paths depend on the relative speeds and directions of move-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W
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FIG. 7. Maps of tracks of fish no. 5–10. Symbols as in Fig. 6.

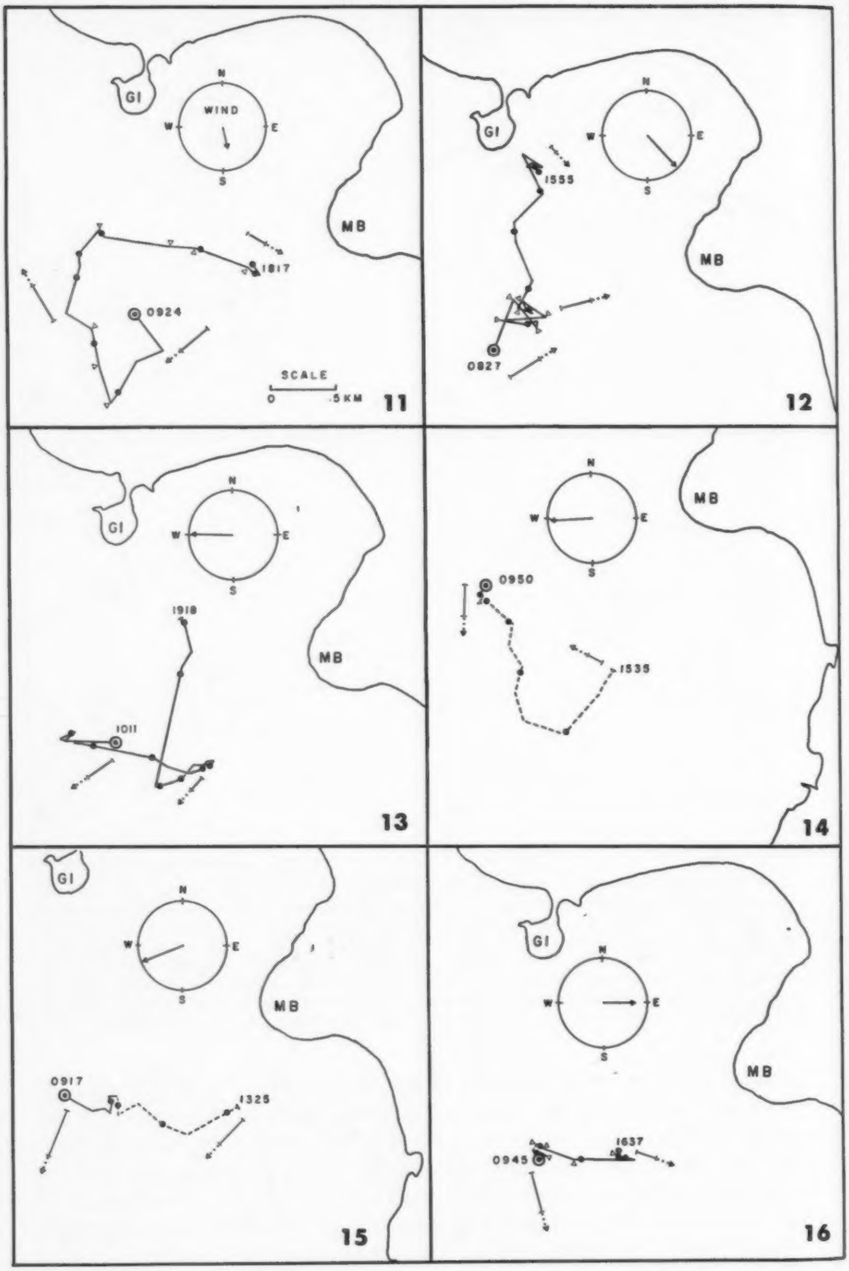
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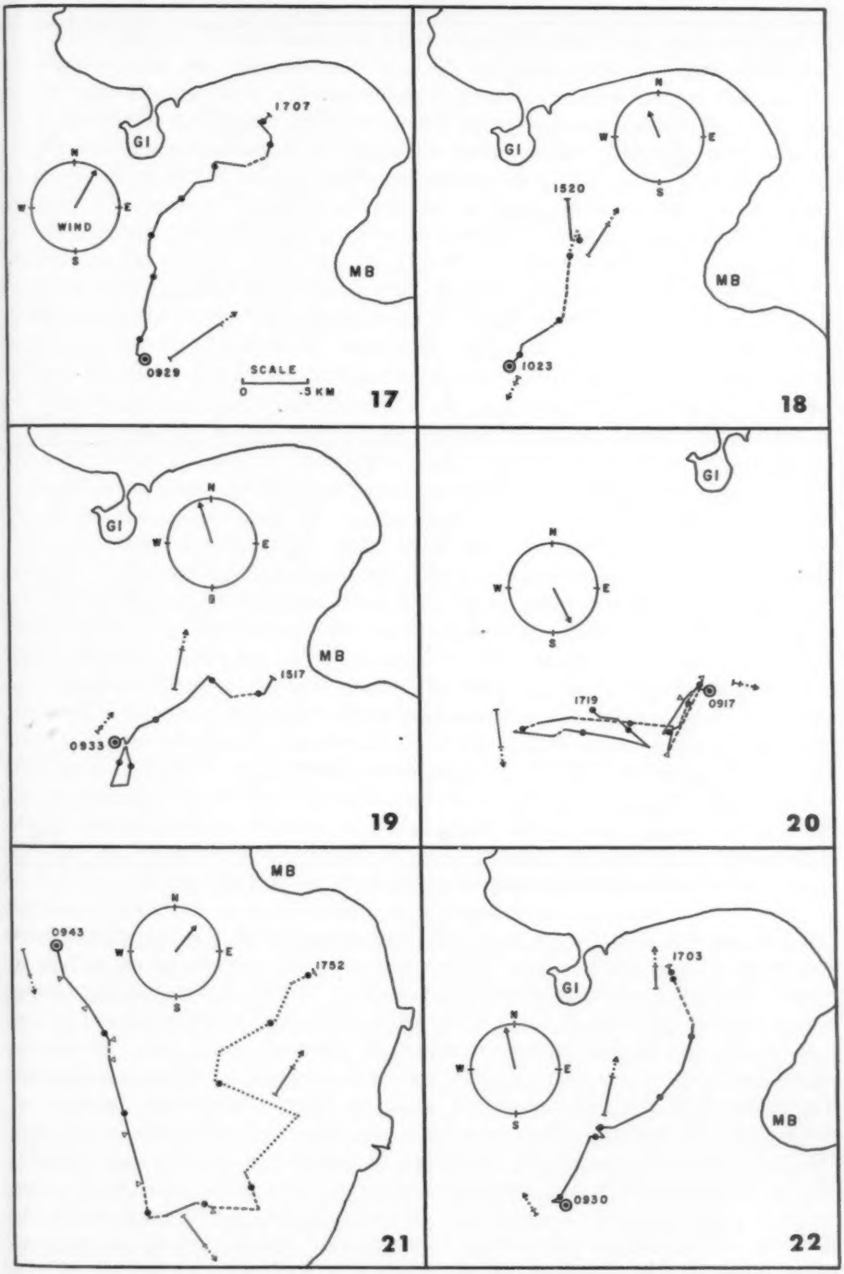
FIG. 8. Maps of tracks of fish no. 11–16. Symbols as in Fig. 6.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20
MB
N
W
E
S
0943
1752
21
1703
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FIG. 9. Maps of tracks of fish no. 17–22. Symbols as in Fig. 6. Fish 18 was the first fish tracked during the post-spawning period.

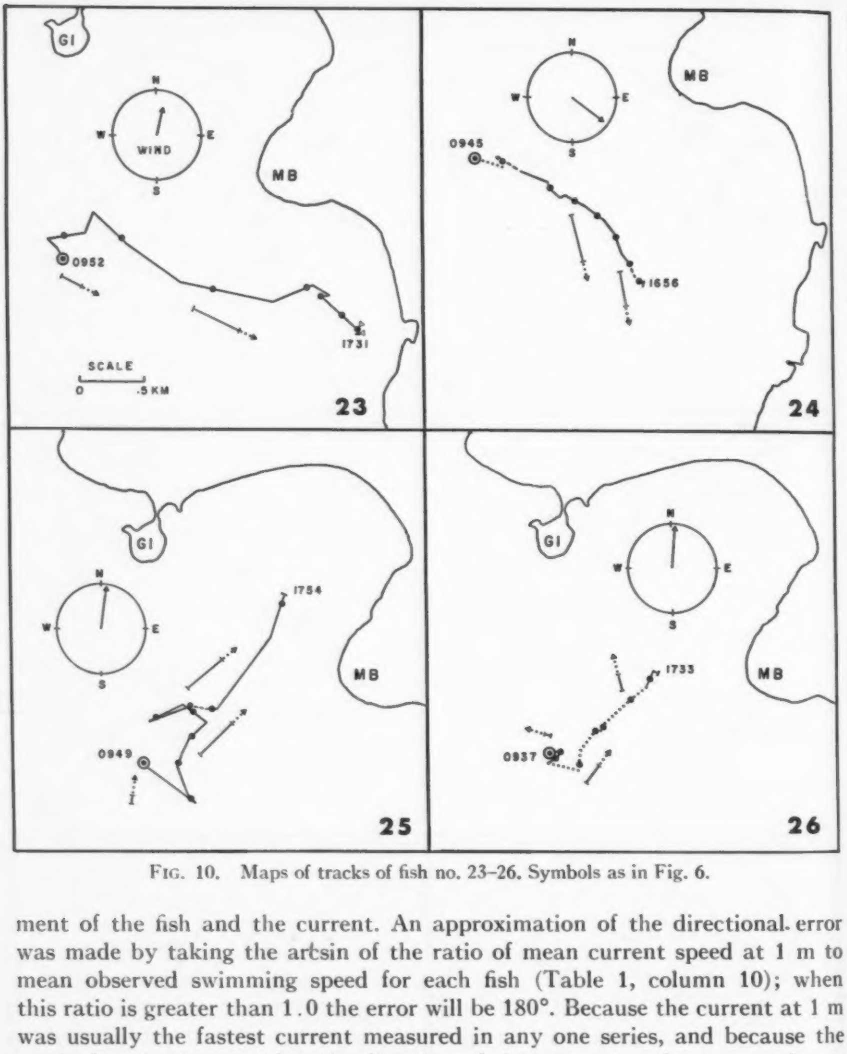

FIG. 10. Maps of tracks of fish no. 23–26. Symbols as in Fig. 6.

ment of the fish and the current. An approximation of the directional error was made by taking the arcsin of the ratio of mean current speed at 1 m to mean observed swimming speed for each fish (Table 1, column 10); when this ratio is greater than 1.0 the error will be 180°. Because the current at 1 m was usually the fastest current measured in any one series, and because the arcsin function insures that the direction of the current produces a maximum displacement in the fish's observed path, we have a maximum estimate of mean error for direction. If one excludes the three fish where the estimate is 180°, the average error for the remaining 23 fish is only 28.8° (range 12–60°). We feel these are fairly good maximum estimates provided the fish were swimming at a depth of 1 or more meters. These estimates could be greater if the fish were swimming at the surface where current speeds can be as much as twice those at 1 m (Lathbury et al., 1960; and Shulman and Bryson, 1961).

Although we do not know at what depth the fish were swimming, it is of interest that the observed paths of six spawning and five post-spawning fish (Table 2, column 2) corresponded roughly to the direction of the current at 1 m. The criterion for correspondence used here was subjective, although where such correspondence occurred the mean current direction was usually no more than 50° from the mean swimming direction. Fish 11 and 21 are noteworthy because directional changes in their paths were accompanied by similar directional changes in the current at 1 m. With the possible exception of four of the tracks (no. 1, 17, 24, and 26) in which passive drift may have been important, these correspondences suggest that the fish may be actively orienting to the current in the downstream direction. It has not been demonstrated, however, that fish can sense current direction while swimming in the pelagic region of a lake where fixed reference points are presumably absent.

One cue associated with direction of the current is the wind-generated surface waves. Schwartz and Hasler (1966a, b) have demonstrated under laboratory conditions that *Fundulus notatus* and *Umbra limi* are able to make directional responses to surface waves using their lateral-line sensory system. These surface waves were, of course, smaller than wind-generated waves on lakes. In addition, Henderson (MS, 1963) hypothesized that fish may orient visually to the subsurface light rays which are produced by and move with wind-generated surface waves. Therefore, mean wind directions (shown with each track), as correlates with wave direction, were compared with the observed paths of the fish on those days when surface waves were present for more than 90% of the entire track. The criterion for correspondence here was also subjective, although where such correspondence occurred the mean wind direction was usually no more than 50° from the mean swimming direction. Nine paths (Table 2, column 3) corresponded with wind direction, but, with the exception of fish 18 and 26, there was also a correspondence to the direction of the current at 1 m. On several days surface waves were absent for at least part of the track (symbolized by an open triangle in the figures and listed in Table 2, column 4), but no consistent changes in directionality of the track between smooth vs. rough surface conditions were apparent.

White bass may use a sun-compass mechanism for open-water orientation (Hasler et al., 1958). There were 16 days where the sun was visible or intermittently visible throughout the tracking period. In the remaining 10 days the sun was completely obscured by clouds long enough to include at least three position determinations (Table 2, column 5; Fig. 6–10). The criteria for sun visibility were based on human vision and, therefore, may not be a true index of what the fish sees in water. There was only one example (fish 5, Fig. 7) where sky conditions (low, heavy overcast) caused the sun to be continuously invisible throughout the track. There was a large southern component during the first part of this track, but the fish subsequently headed northeast on a fairly steady course and finally arrived "home" at Maple Bluff. In the remaining nine examples sun visibility varied during the track.

These tracks were examined for changes in orientation behavior which might be related to changes in sun visibility. The paths of fish 1, 3, and 21 showed no consistent directional changes corresponding to changes in sun visibility. Fish 26 remained near the release point for 2.5 hr while the sun was visible, but then, when clouds obscured the sun, took a steady northeasterly course. In contrast to the above examples, there were tracks where the sun appeared to influence the path. The sun was obscured during the first portions of tracks 6 and 9. As the sun became visible — intermittently at first for fish 9, continuously for fish 6 — the fish altered their courses and each maintained a new course for the remainder of the track. Looping movements of fish 18 coincided with a brief period when the sun was obscured. Fish 4 and 22 were in shallow water while the sun was obscured and, hence, the effect of the sun's absence cannot be evaluated in terms of open-water orientation. Although these variable results certainly do not rule out the sun as an orientation cue when it is available, they do suggest that, in the absence of the sun, white bass may use other undetermined cues for maintaining direction.

Significance of Findings

Because of the similarities between the spawning and post-spawning tracks one might hypothesize that any Lake Mendota white bass, regardless of reproductive condition, moves easterly upon finding itself in an unfamiliar open-water location. An alternative hypothesis is suggested by the observation that there was a greater percentage of post-spawning than spawning tracks corresponding to wind and current directions. If nonspawning white bass are indeed influenced more by these highly variable environmental factors, and spawning fish rely mostly upon some more stable cue, such as the sun, for example, one could argue that the similarities between the two 1965 series arose because the distributions of current and wind directions during the post-spawning period corresponded by chance to the distribution of the spawning-season tracks.

The present study has confirmed the findings of Hasler et al. (1958) that the open-water orientation of displaced white bass is nonrandom with a directional bias toward the spawning grounds. One discrepancy between these studies was that fish tracked ultrasonically remained randomly distributed up to 1 or 2 hr after release, whereas in the float-tracking experiments the fish were distributed nonrandomly at the end of 1 hr. Differences in sample size, environmental factors, and tracking methods may all have contributed to this discrepancy. It is possible that the additional 2 g weight of the transmitter may have affected the initial random movements of the fish; this initial period could represent the time required for the fish to regain hydrostatic equilibrium.

Whereas the float-tracking experiments showed only the initial movements of the fish, ultrasonic tracking has extended tracking time and provided a more complete knowledge of the homing process in displaced white bass. After their release in open water the displaced fish assumed a steady course or a series of steady courses until they reached the shallow littoral zone of the lake. The observed tracks rarely took the fish directly to the spawning ground at Maple Bluff, but there was sufficient directional bias to bring fish into the littoral zone somewhere near the spawning area. Once in the littoral zone presumably additional information concerning location would be available.

Although this study has demonstrated that white bass can make prolonged oriented movements in open water, the mechanisms underlying this ability are still obscure. The initial and simplest approach for studying the problem of mechanisms is to test hypotheses based on single cues and single sensory systems. It may be, though, that white bass utilize combinations of cues and senses simultaneously or perhaps in the absence of one cue are able to switch to others. Whichever hypothesis one may choose to examine, it is clear that information concerning swimming depth is desirable. It is also abundantly clear from the present study that due to the variable nature of the fish's open-water movements, large numbers of fish will have to be tracked before it will be possible to draw any significant conclusions from tracking studies concerning the mechanisms of orientation in this species.

ACKNOWLEDGMENTS

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Effect of the Organophosphate Dylox on Rainbow Trout Larvae

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MATTON, PIERRE, AND QUENTIN N. LAHAM. 1969. Effect of the organophosphate Dylox on rainbow trout larvae. J. Fish. Res. Bd. Canada 26: 2193–2200.

Organophosphate poisoning in fish and mammals has always been associated with the inhibition of acetylcholinesterase in both neuromuscular and brain tissues. This inhibition resulted in the apparent death of the organisms by asphyxia. Rarely have other effects been reported.

In the present study, 1-inch rainbow trout larvae were treated for 16 hr with from 10 to 100 ppm Dylox, or for 40 hr with 5 ppm. This treatment produced a marked acetylcholinesterase inhibition that was reflected by their abnormal behaviour patterns. Histological examination revealed pathological changes in the heart, liver, blood cells, pseudogills, and muscular tissues; this pathology could not be explained on the basis of acetylcholinesterase inhibition.

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L'empoisonnement des poissons et des mammifères par les organophosphorés a toujours été attribué à une inhibition de l'acetylcholinesterase du tissu nerveux et des jonctions neuromusculaires. L'asphyxie résultant de cette inhibition causait la mort, et des effets autres que cette inhibition n'ont été observés que très rarement.

Au cours de cette étude, nous avons soumis des alevins de truites arc-en-ciel d'un pouce de long à des concentrations de Dylox variant de 10 à 100 ppm (parties par million) pendant 16 hr. et à une concentration de 5 ppm pendant 40 hr.

Il en résulta une inhibition importante de l'acetylcholinesterase illustré par le comportement anormal des alevins. Des coupes histologiques montrèrent que le coeur, le foie, les érythrocytes, les pseudobranchies et en particulier les muscles avaient subi des changements pathologiques qui ne peuvent être expliqués par l'inhibition de AchE.

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INTRODUCTION

BECAUSE OF THEIR LOWER TOXICITY in both fish (Kerswill and Edwards, 1967) and mammals (Casida, 1964), as well as the property of being rapidly degraded to harmless products, organophosphates are gradually replacing chlorinated hydrocarbon insecticides.

Organophosphate poisoning in mammals is associated with a decrease in cholinesterase activity. Death in these animals has been attributed to asphyxia caused by cholinesterase inhibition (Heath, 1961; O'Brien, 1960;

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Casida, 1964). In fish, the results were not as definitive; however, some cholinesterase inhibition (40–70%) has occurred (Weiss, 1958, 1959, 1964, 1965). Weiss does not speculate on the cause of death in fish and he does not record any disorders other than the inhibition of cholinesterase. This would suggest that organophosphates cause fish mortality because of the inhibition of cholinesterase.

The current study on trout larvae was undertaken to show whether organophosphates have other effects besides cholinesterase inhibition.

MATERIALS AND METHODS

Rainbow trout eggs were obtained from the Coaticook hatchery in Quebec. After the larvae reached the stage where the yolk mass was half resorbed, they were divided into groups of 150. Each group was placed in separate hatching trays and kept in a cold room at 10 C. Five gallons of water were continuously recirculated through these trays at a rate of 2 gal per minute. The *p*H (7.4) and the dissolved oxygen concentration (12 ppm) of the water was unchanged during the experimentation. Each group was treated for 16 hr with 10, 30, 50, and 100 ppm of Dylox (50% soluble powder supplied by Chemagro Corp., Kansas City, Missouri) and for 40 hr with 5 ppm. After this treatment, the larvae were allowed to recover in pre-cooled aerated water.

The following criteria were studied.

1. Larvae Behaviour

The larvae were observed at 6, 10, 16, 32, 40, and 76 hr after the onset of treatment. The parameters studied included (*a*) the spontaneous activity of the larvae; (*b*) their reaction to light and to a touch stimulus; (*c*) their heart rate and per cent mortality.

2. Acetylcholinesterase Activity

Larvae samples for the histochemical determination of acetylcholinesterase were removed after 6, 16, and 32 hr of treatment with Dylox. These larvae were fixed in formol saline for 1 hr at 4 C. After fixation, the larvae were immersed in gum sucrose at 4 C for 16 hr, and embedded in O.C.T. (Optimun Cutting Temp Compund) (Labtek). Eight micra cryostat sections were stained for acetylcholinesterase according to the method devised by Karnovsky and Roots (1964). The sections were incubated at room temperature for 45 min. The acetylcholinesterase activity of the treated larvae was estimated by comparing the intensity of the reaction with the untreated control. The reaction was also compared by varying the incubation time of the experimental larvae section so as to obtain colour intensities equal to those of the controls.

3. Histological Examination ,

Larvae, treated for 10 hr in Dylox, were fixed in Bouin's solution and embedded in paraffin. Ten micra sections were stained with haematoxylin-phloxine-orange G for microscopic examination.

RESULTS

Larvae Behaviour

Before Dylox treatment, the larvae were in a normal resting position with the yolk pouch touching the bottom of the tray. The larvae aggregated at the shadowed corners of the trays. At various intervals, the larvae swam distance of 6–10 inches and then rested.

SPONTANEOUS ACTIVITY

After exposure to the organophosphate, the larvae became hyperactive (they swam very fast and erratically for short distances and often changed direction). After this initial activity, the larvae became hypoactive until eventually they came to rest on their sides.

REACTION TO LIGHT AND TOUCH STIMULI

The larvae no longer avoided light, and their response to the touch stimulus became weaker and retarded. Normal swimming activity gave way to a jerky forward movement that culminated in a jump produced by a single long-lasting contraction of the body. Later, the animals could no longer jump, but instead simply curled their bodies. In the final stages there was no visible response; however, upon stereoscopic examination continuous fascicular twitching was evident.

The various phenomena of behaviour were independent of the Dylox concentration, but the time lapse between the changes became more rapid with an increase in the concentration. For example, after 6 hr, the larvae treated with 10 ppm Dylox were hyperactive and responded strongly to a stimulus, whereas larvae treated for 6 hr with 50 ppm lay on their sides and were unable to respond to a touch stimulus.

HEART RATE AND MORTALITY

The mortality and heart rate data are given in Table 1. At concentrations above 10 ppm the animals died. The heart rate and strength of contraction decreased markedly especially when the animals lay on their sides (final stage of the poisoning as previously described).

ACETYLCHOLINESTERASE ACTIVITY

Cryostat sections from control larvae display moderate acetylcholinesterase activity in the optic lobes of the midbrain and in the plexiform layer of the retina. The activity was strong in the ganglia, gray matter of the neural tube, at the myoneural junctions, and at the septa of the myotomes of the body musculature.

Sections from Dylox-treated larvae displayed acetylcholinesterase activity in the same areas as above but the intensity of the staining was weaker. An estimate of the acetylcholinesterase activity in the treated larvae is given in Table 2. The acetylcholinesterase activity in the nervous system, in the septa of the myotomes, and at the myoneural junctions appeared to be inhibited at the same rate.

HISTOLOGICAL EXAMINATION

Paraffin sections from larvae treated for 10 hr were examined histologically. The affected areas included the pseudogills, the circulatory system, and skeletal

TABLE 1. Heartbeat[a] (H.B.) rate and percentage mortality (Mort.) in rainbow trout larvae during and after treatment with Dylox.[b]

Dose (*ppm*)		Time (*hr*)					
		6	10	16	32	40	76
0	H.B.	*s* 120	*s* 120	*s* 120	*s* 120	*s* 120	*s* 120
	Mort.	0	0	0	0	1	1
5	H.B.	*s* 120	*s* 120	*s* 120	*s* 80	*s* 80	*m* 80
	Mort.	0	0	0	1	3.5	4
10	H.B.	*s* 120	*s* 120	*m* 90	*s* 106	*s* 120	*s* 106
	Mort.	0	0	0	0	0	1.5
30	H.B.	*m* 106	*m* 40–95	*m* 89	*s* 110	*s* 70	*s* 95
	Mort.	0	1	27	45	50	51
50	H.B.	*m* 110	*w* 50	*w* 60	*s* 120	*s* 80–120	*s* 120
	Mort.	0	0	61	78	79	79
100	H.B.	*w* 54	–				
	Mort.	71	100				

[a] The strength of contraction was classified as strong (*s*), medium (*m*), or weak (*w*).

[b] Treatment with 5 ppm lasted for 40 hr whereas treatment with 10, 30, 50, and 100 ppm lasted for 16 hr. After treatment the trout larvae were allowed to recover in pre-cooled aerated water.

TABLE 2. Estimate of acetylcholinesterase activity (%) in rainbow trout larvae during and after Dylox treatment.[a]

Dose (*ppm*)		Treatment time (*hr*)			Recovery time (*hr*)
		6	16	32	16
0	Control	100	100	–	100
5		75	50	20	–
10		50	30	–	40
30		40	20	–	40
50		25	15	–	25

[a] Treatment with 5 ppm lasted for 40 hr whereas treatment with 10, 30, 50, and 100 ppm lasted for 16 hr. After treatment the trout larvae were allowed to recover in pre-cooled aerated water.

musculature. The relationships between the extent of the pathology and the concentration of Dylox administered are given in Table 3.

PSEUDOGILLS

The pseudogills, found in the posterior dorsolateral part of the mouth, are normally arranged in parallel rows of three layers per row. The two exterior

TABLE 3. Histological damage[a] in rainbow trout larvae after 10 hr of Dylox treatment.

Tissue	Dose (*ppm*) 5	10	30	50
Blood	–	–	+	+
Heart	–	–	+	+
Pseudogills	+	+	++	++
Red fibers	+	++	+++	++++
White fibers	–	+	+++	++++

[a] – signifies no damage; + to ++++ signifies relative damage from little (+) to extensive (++++).

layers contain elongated narrow cells and the midlayer is a single row of blood cells. In the treated animals this arrangement became disrupted; the cell rows were no longer parallel, the elongated epithelial cells were distended with swollen nuclei, and distorted blood cells were present in the midzone (Fig. 1 and 2).

CIRCULATORY SYSTEM

In the treated larvae extravasated blood was often present. Blood vessel walls appeared distorted rather than ruptured. Blood cells were irregular and shrunken. The cytoplasm of some cardiac muscle cells was vacuolated and some fibers were streaked.

SKELETAL MUSCULATURE

Extensive histological damage was evident in both the red and white muscle fibers.

White muscle — White muscle constitutes the main mass of the body musculature. In the treated larvae some of these fibers appeared stretched and the cytoplasm was clear; other fibers were swollen and glossy; fibers which appeared structurally normal were markedly contracted (Fig. 3). In cross-section the myofibrils were grouped in the center of the cells and were surrounded by granular material containing large oval distended nuclei and some myofibrils were fused (Fig. 4 and 5). At high concentrations some fibers were completely broken down to an amorphous granular material with free nuclei (Fig. 6 and 7).

Red muscle — The small red fibers of the ocular (most severely damaged) masseter, and cardinalis muscles as well as in the muscles of the pharynx, esophagus, and anterior part of the stomach were damaged extensively. These fibers were either completely disrupted, reduced in diameter, or swollen; all lacked striations (Fig. 8–10).

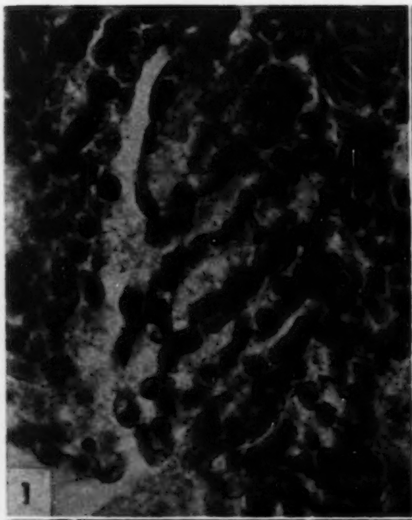
DISCUSSION

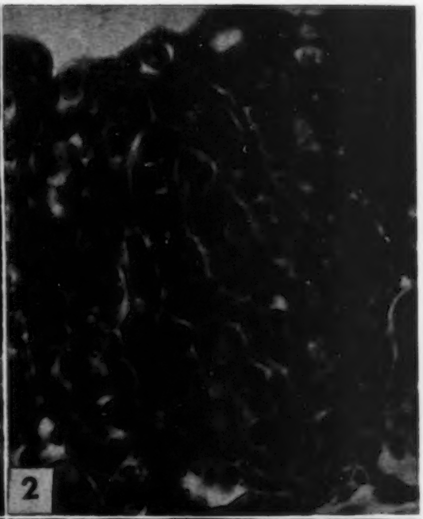
The anticholinesterase property of Dylox on trout larvae is evident from histochemical and behaviour studies found elsewhere in this report. The anticholinesterase activity is not due to the Dylox per se, but is probably due to the transformation of Dylox (*o,o,*dimethyl,2,2,2,-trichloro-1-hydroxy ethylphosphonate) into DDVP (*o,o,*dimethyl,2,2,-dichloro vinyl phosphate) by dehydrochlorination in an alkaline aqueous solution (Lorenz et al., 1955; Van Die, 1957). Mammals rapidly metabolize Dylox before it can be transformed into DDVP; this accounts for its low toxicity in these animals (Robbins et al., 1956). From our results on trout larvae, the formation of DDVP would explain the inhibition of acetylcholinesterase but not the tissue pathology.

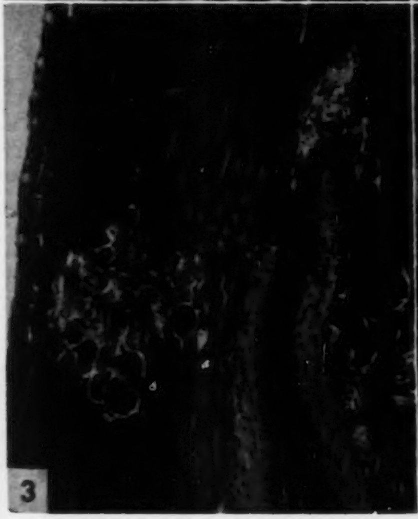
The LC_{50} values were in agreement with those obtained by Willford (1967). From Tables 1 and 2, there is no correlation between the per cent mortality and acetylcholinesterase levels. For example, after 32 hr of treatment with 5 ppm about 1% of the larvae were dead whereas treatment for 16 hr with 50 ppm caused 61% to die; the acetylcholinesterase levels of both groups were about equal.

Other workers have seldom reported histological damage as an effect of organophosphate poisoning. Abnormalities have been reported that are not unique to organophosphate exposure but that could be due to disturbances in acetylcholinesterase metabolism or hypoxia (demyelination in chicks (O'Brien, 1960), dystrophic and necrobiotic changes in liver, kidneys, myocardium, and endocrine glands after lethal doses of mercaptophos and disistone in rats and cats (Kagan and Makovskaya, 1960)). In our experiments, disturbances in the heart tissue (and occasional vacuolation of the liver cells) could have been caused by tissue hypoxia because of the decrease in the heart rate and strength of contraction and a decrease in the activity of the opercular muscle. The damage to the blood cells and pseudogills suggests an electrolytic imbalance that produces an increase in the permeability of the cell membranes.

Striated muscles are resistant to chemicals (Field, 1960; Adams et al., 1962) except for the degeneration induced by plasmocid (Hicks, 1950) and vincristine (Slotwiner et al., 1966). Khera et al. (1966) have reported muscle damage in hatched ducks that were injected with EPN at the 13th day of incubation. The dystrophic changes in these muscles were characterized by fiber fragmentation and proliferation and central displacement of sarcolemmal nuclei; these changes could be because of a lack of proper differentiation rather than a positive degeneration. The muscular dystrophy in our trout larvae appears similar to the degeneration caused by plasmocid such as the formation of hyaline material, swelling of segments and loss of cross and longitudinal striations. The muscle degeneration in trout larvae may be a result of muscle fatigue due to continous contractions. The muscle ultimately becomes exhausted, dies, and degenerates. This degeneration could, however, result from the direct action of Dylox on the components or metabolic pathways, or both, of the muscles themselves.







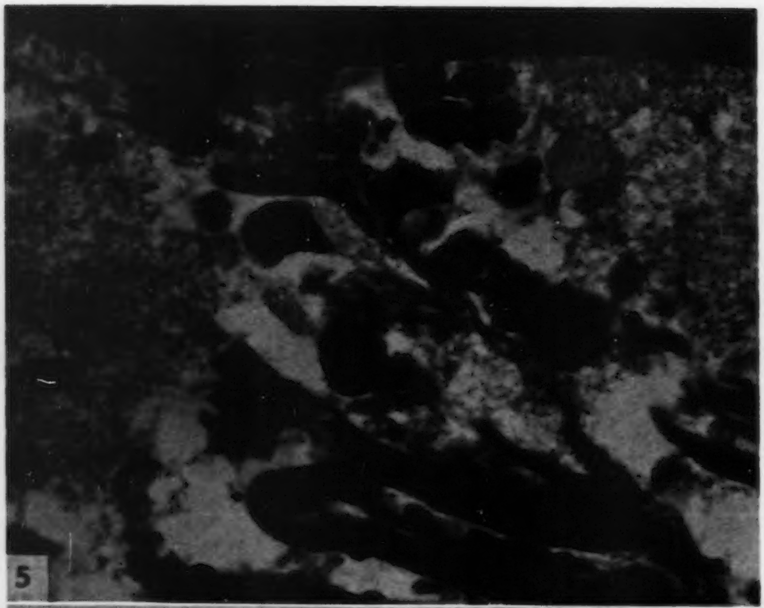
FIGURES 1 to 10 are photomicrographs of paraffin sections (10 μ) stained with haematoxylin-phloxine-orange G.

FIG. 1. Dylox-treated (50 ppm for 10 hr) trout larva illustrating disrupted pseudogills (500×).

FIG. 2. Control larva illustrating normal pseudogill structure (500×).

FIG. 3. A longitudinal section of the tail in Dylox-treated larva (50 ppm for 16 hr, 16 hr of recovery). Notice an area of swollen glossy fibers (125×).

FIG. 4. A cross-section of white fibers in Dylox-treated larva (50 ppm for 10 hr). Note the large amount of peripheral cytoplasm (1250×).



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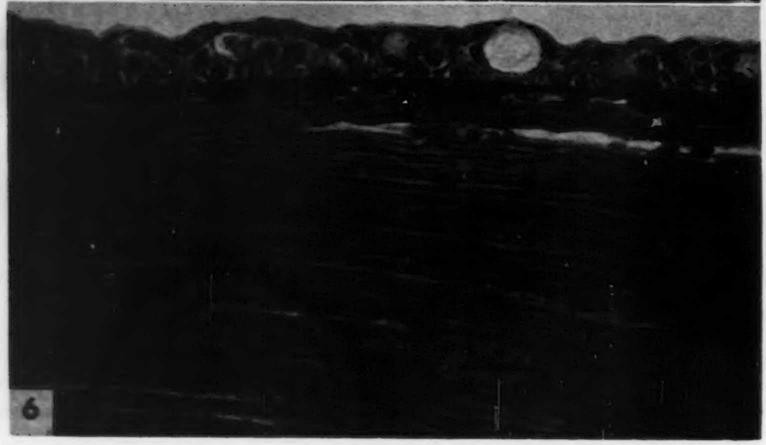

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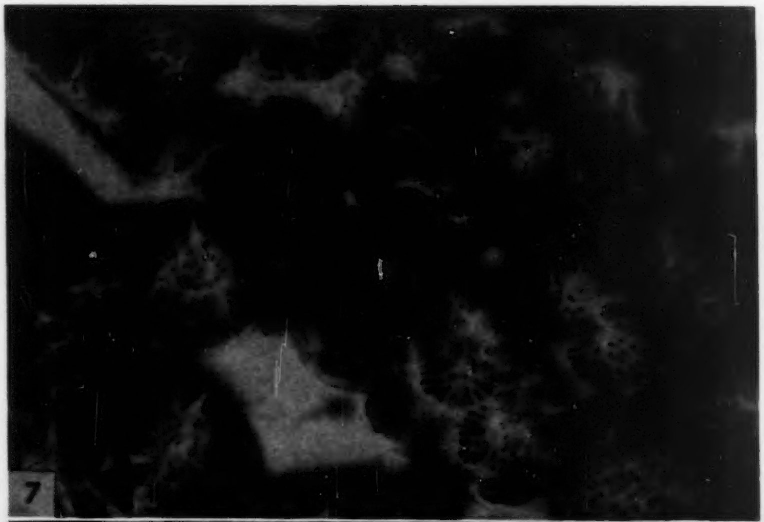
FIG. 5. Swollen white fibers in the tail of a Dylox-treated larva (50 ppm for 10 hr). Observe the amorphous granular material containing free nuclei (500×).

FIG. 6. A longitudinal section of normal white fibers in the tail of a control larva (500×).



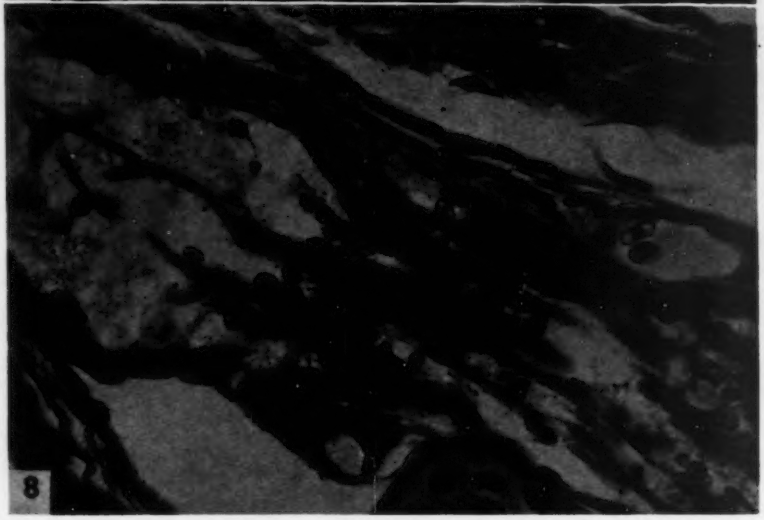
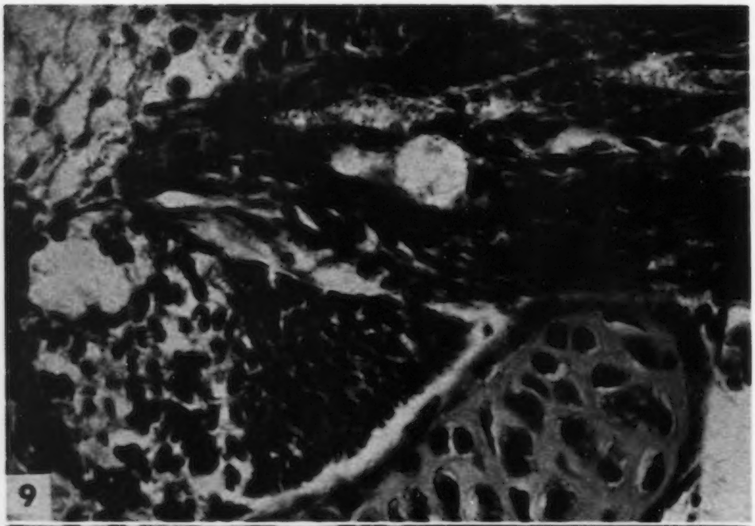


FIG. 7. Abnormal white fibers in Dylox-treated larva (50 ppm for 10 hr). The myofibrils are being fused into a homogenous mass (1250×).

FIG. 8. Ocular muscle in Dylox-treated larva (50 ppm for 10 hr). The fibers are thin with stretched segments and dense swollen areas. Observe the free nuclei in the granular matrix (500×).



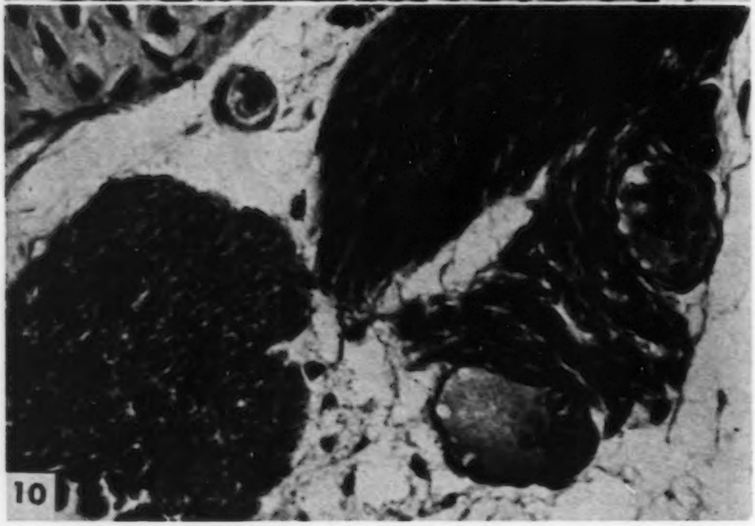


FIG. 9. Ocular muscle in Dylox-treated larva (50 ppm for 10 hr). Several of the muscle fibers are degenerating (500×).

FIG. 10. Normal ocular muscle in control larva (500×).

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Durham et al. (1957) have noted that animals given DDVP could survive with a cholinesterase activity less than 20% of the normal. In our experiments, high mortality is associated with tissue damage but the acetylcholinesterase activity is low in both groups. Factors (unknown) other than cholinesterase inhibition must be involved in the poisoning of rainbow trout larvae by the organophosphate Dylox.

ACKNOWLEDGMENTS

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NOTES

Tagging White Whales in the Canadian Arctic

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White whales, *Delphinapterus leucas* Pallas, were tagged in the estuary of Seal River, Man., in western Hudson Bay in the summers of 1967 and 1968. Seven hundred harpoon tags were applied to the dorsal part of the body while chasing the animals, and 118 Petersen disc tags through the dorsal ridge after stranding the animals in shallow water on a falling tide. Three recovered harpoon tags include one observed on a living whale at the same site after a year, and two taken from netted whales at fisheries northward along the western coast of Hudson Bay, 300–800 km from the site of tagging and 5–7 weeks after tagging.

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TWO COMMERCIAL FISHERIES for white whales or beluga (*Delphinapterus leucas* Pallas) exist in western Hudson Bay (Fig. 1). The first, located at Churchill, Man., supplies harpooned white whales to a small whaling plant (Doan and Douglas, 1953). The second is a net fishery located at Whale Cove, Northwest Territories, some 350 km to the north, which supplies "muktuk" (an Eskimo delicacy composed of skin, with a little fat) and meat for canning in a Government-supported industry maintained for the benefit of the local Eskimo population.

We sampled the catch at both sites and found that the two sets of animals have similar length distributions, reproductive seasons, and other biological attributes, and thus give no evidence of being separate populations. White whales in western Hudson Bay are smaller than animals occurring in Davis Strait, suggesting that they remain isolated in Hudson Bay all year (Sergeant and Brodie, in preparation). However, aerial surveys along the coast north

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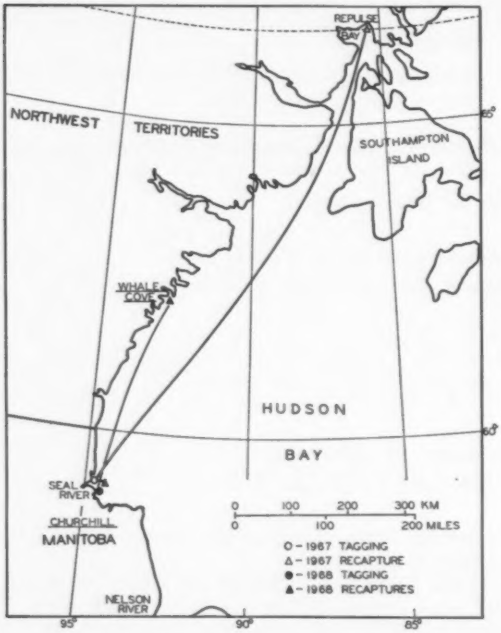
REPULSE BAY
NORTHWEST TERRITORIES
65°
SOUTHAMPTON ISLAND
WHALE COVE
HUDSON
BAY
60°
SEAL RIVER
CHURCHILL
MANITOBA
NELSON RIVER
0 100 200 300 KM
0 100 200 MILES
O - 1967 TAGGING
Δ - 1967 RECAPTURE
● - 1968 TAGGING
▲ - 1968 RECAPTURES
95°
90°
85°

FIG. 1. Principal locations of hunting (underlined) and sites of tagging and recoveries of white whales in western Hudson Bay, 1967, 1968.

and south of Churchill at different times in the summer have thrown no clear light on seasonal movements, except that all white whales leave this area by early September.

Previous tagging experiments by Doan and Douglas (1953) and by Sergeant using tattoos were unsuccessful. The purpose of this study was thus to study migrations and relations of stocks by renewed tagging using new techniques. The paper reports results in the two years 1967 and 1968.

Materials and methods — Recent tagging of dolphins in aquaria (Nishiwaki et al., 1966) suggested, as possible tagging methods, harpoon tags and Petersen type discs. Harpoon tags seemed easy to apply, but Nishiwaki's experiment suggested that they would not remain attached for more than a few weeks or months. However, since the fisheries at Churchill and at Whale Cove are separated in time by only a few weeks, we thought that even if tags stayed in for short periods some information on migrations would be gained. Since the white whale has no dorsal fin, the use of Petersen-type fish tags seemed less convenient than for the true dolphins, but we decided to try to apply them in the dorsal ridge.

The available Petersen-type tags, designed for salmonid fish, consisted of plastic discs 13 mm in diam, pierced for a headed pin of monel metal 7 cm long. To apply the tag, the end of the pin opposite the head is twisted with pliers against the outside of the second disc.

The harpoon tags (Floy Tagging Co., Seattle, Washington) are of a type (FH-67) made initially for the Cooperative Game Fish Tagging Program of the Woods Hole Oceanographic Institution (Mather, 1963). They have a head of monel metal, and the shaft is a 15-cm long, flexible, cylindrical rod of nylon, the proximal end of which is pierced through the metal head and heat-sealed in the form of a bead. The distal seven-eighths of the shaft is covered with a sleeve of bright coloured plastic on which is stamped the return address, the word "Reward," and the serial number. These tags were mounted on an applicator described and figured by Mather (1963), and set into a simple hand harpoon of Eskimo design. The applicator protruded sufficiently to set the harpoon tags about 5 cm into the integument of the whale.

In case of shearing due to forward movement of the animals harpooned from above, a simple breaking device was initially built into the harpoon, again using an Eskimo design. A simple harpoon without the breaking device eventually proved adequate for rapid application of tags.

As with the tags used at Woods Hole, the address was stamped into the coloured plastic sleeve of the harpoon tags and left uncovered.

We selected as a site the estuary of Seal River, 40 km north of Churchill. Although this river is small, its estuary is frequented in summer by up to 1500 white whales. From previous aerial surveys and a visit to the locality we knew that the largest numbers were to be found here through July and the first part of August, and that suitable shallow water free of rocks existed where hopefully we could drive the animals ashore.

In 1967 we worked Seal River from August 1–4 with 10 canoes each manned by two local hunters. Nine of the canoes were 22-ft (7-m) "Voyageurs" fitted with outboard motors (20–22 hp). These are short and broad and capable of quick turning. The 10th was a longer, speedier, but less maneuverable freighter canoe. In 1968 the number of canoes was reduced to six.

The driving technique was adapted from the hunting of pilot whales, *Globicephala melaena*, in Newfoundland (Sergeant, 1953). We found that the head of the estuary formed a basin about 2 km long by 1 km broad at low tide, having then a depth less than 2 m and a bottom of hard sand almost entirely free of the fields of rocks, exposed at low tide, which lay everywhere around it along the coast. This basin, moreover, was nearly blocked at the seaward end by an esker ridge of rocks having one main and two subsidiary exits. Many whales entered this basin voluntàrily; others were slowly driven in by a crescent of canoes, on a falling tide. A few canoes guarded the exits until near extreme low tide while other canoes stranded groups of whales on the sand at the sides of the basin, giving us about 1 hr to tag them (Fig. 2). The whales proved docile and could be tagged in the dorsal ridge, so long as they were hard aground. If partly buoyant, they propelled themselves forward and escaped. On the last day of the 1967 operation we had some 500 white whales massed in this shallow water, 50 of which were stranded. We were limited by

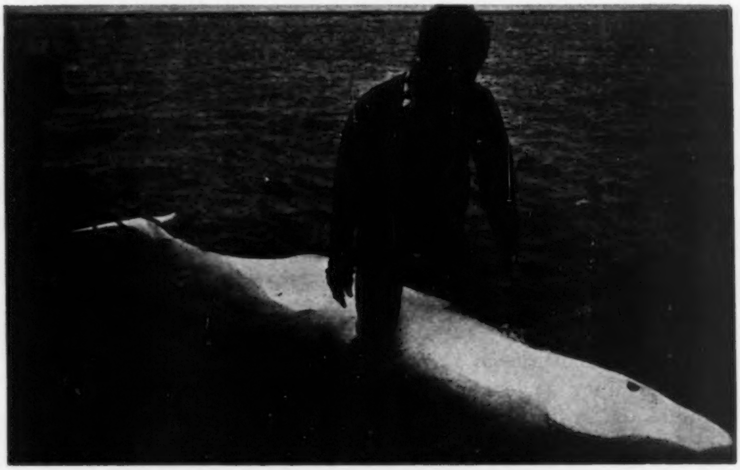

FIG. 2. A stranded white whale. (Photo by Charles Brill — Galaxy International Ltd.)

the short period of low water, by cold, and by shortage of trained staff, and were able to tag only 45 animals using the disc tags.

The hunters, who are skilled harpooners, had no difficulty applying the harpoon tags into the backs of animals as they surfaced in water of 3-m depth or less, and applied the 100 harpoon tags that were available within two periods of low tide. They found it difficult, however, to identify fresh whales from those already tagged, and some whales carried away two or even three tags, leaving an estimated 94 animals effectively tagged.

We repeated the experiment between July 30 and August 6, 1968, when we applied 600 harpoon tags and 73 disc tags in 3 days of fine weather using much the same techniques as the previous year. Thus, harpoon tags were attached to 694 animals and disc tags were fastened to 118 during the 2 years.

In addition, we gave intramuscular injections of tetracycline to 73 animals tagged with disc tags and to 20 tagged with harpoon tags, making the injection dorsal to the flipper, and giving dosages proportional to the estimated body weights (approx 20 mg per kg body weight). The purpose of this experiment was to try to determine the absolute rate of deposition of the layers of dentine in the teeth used in age determination (Sergeant, 1959; Khuzin 1963). We advertised an extra reward at Whale Cove, the expected point of recapture, for return of the animal's jaw together with the tag. We have not as yet obtained results from this subsidiary experiment.

Results — All three recoveries to date (November 1968) have been harpoon tags. In each year, one tag was recovered, each from a distant fishery. The first was a harpoon tag taken by an Eskimo hunter from a white whale in a net near Repulse Bay, Northwest Territories, in the last week of September 1967. This was over 800 km from the point of tagging and after a lapse of 7 weeks (Fig. 1).

In 1968, an Eskimo hunter recovered one new harpoon tag from a white whale in a net at Whale Cove, on September 5. In addition we observed a single 1-year-old harpoon tag on a living white whale stranded at Seal River. Complete healing had occurred with negligible epidermal scarring around the point of tagging. The tag was in excellent structural condition. However, the stamped letters were obliterated. This defect has now been remedied by specifying an additional clear plastic sleeve to cover the coloured plastic on which the legend is stamped.

The returns show that white whales migrate long distances in Hudson Bay, the animals moving north along the coast in late summer, so that the fisheries at Churchill and Whale Cove draw upon the same stock. They also show that animals may return to the same site.

No disc tags have been recovered and it seems possible that the animals rub them off, although the ratio of taggings by the two methods, and the small number of recoveries of harpoon tags, does not allow us to rate the disc tags with certainty as less efficient.

We were unable to tag newborn calves with disc tags, owing to the extreme softness of the skin. Nor did we inject these young animals, fearing injury.

Discussion — The use of harpoon tags is likely to be more successful for tagging white whales and narwhals than true porpoises and dolphins because only the former are thick-skinned. A Petersen disc pierced through the dorsal fin seems a more suitable method for the true Delphinidae where the animals can be handled. For free-ranging porpoises it may be necessary to shoot a modified harpoon tag into or through the dorsal fin from a gun.

Acknowledgments — Mr Frank M. Mather III of the Woods Hole Oceanographic Institution, Woods Hole, Mass., Dr K. S. Norris, University of California, Los Angeles, Calif., and Mr David M. Waller of the University of Wisconsin, Madison, Wis., provided background information on tagging of dolphins. Dr J. R. Geraci advised on methods and dosages for tetracycline injection. We are grateful to Mr H. Voisey, Manager of the Hudson's Bay Company at Repulse Bay, and Mr Charles Livingston, Economic Development Officer of the Department of Indian Affairs and Northern Development stationed at Whale Cove, for reporting tag captures. Finally we attribute the success of the tagging program in no small way to the skill and enthusiasm of the hunters employed.

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White Whales Wintering in James Bay

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JONKEL, C. J. 1969. White whales wintering in James Bay. J. Fish. Res. Bd. Canada 26: 2205–2207.

The white whale or beluga (*Delphinapterus leucas* Pallas) has been found wintering in the numerous open leads adjacent to small islands in James Bay. The spacing of the islands apparently allows year-round open water through tidal and wind action in the narrow channels.

JONKEL, C. J. 1969. White whales wintering in James Bay. J. Fish. Res. Bd. Canada 26: 2205–2207.

On a trouvé des marsouins blancs ou bélugas (*Delphinapterus leucas* Pallas) qui hivernaient dans les nombreux passages ouverts avoisinant des ilôts dans la baie James. C'est apparemment l'écartement des ilôts avec les mouvements de la marée et des vents dans les passages étroits qui permettent aux eaux de demeurer ouvertes à l'année longue.

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THE WHITE WHALE OR BELUGA (*Delphinapterus leucas* Pallas) is abundant in Hudson Bay during the summer as far south as Winisk (Fig. 1) along the western coast and Great Whale River and the Belcher Islands on the eastern coast (Sergeant, 1968). They are commonly observed feeding on fish in the estuaries of smaller streams, in bays near the mouths of rivers, and even considerable distances upstream in the large rivers. White whales from Cape Churchill and areas to the north of Churchill have been observed moving northward along the coast in autumn, and Sergeant (1968) reports white whales during the winter in northwestern Hudson Bay in the wide offshore lead associated with the predominately northwesterly winds of that area. He suggests that there may be an autumn migration of this "ice-adapted" resident of Hudson Bay into or through Hudson Strait, as large numbers of white whales have also been observed in the vicinity of Salisbury and Nottingham Islands during October.

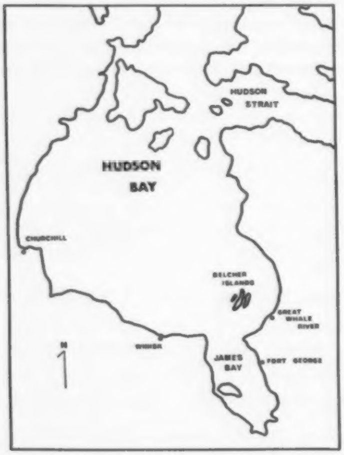

FIG. 1. Hudson Bay and James Bay showing the islands and settlements where white whales have been observed.

On April 2, 1969, I flew by helicopter across James Bay from Fort George, Quebec, to Spencer, North Twin, Akimiski (southeast tip), Weston, South Twin, and Walter islands, and back to Fort George. We were travelling about 10–30 m above the ice searching for polar bears (*Ursus maritimus* Phipps) to capture and mark as part of the Canadian Wildlife Service Polar Bear Project. Three herds of white whales were seen along the route; a herd of 10 and a herd of 5 between Spencer and North Twin Island, and a herd of at least 3 between North Twin Island and Akimiski Island. On April 4, during a similar flight, I saw about 50 belugas off the eastern tip of Grey Goose Island. All herd estimates are minimal, as we were travelling in direct lines and counted only those whales observed as we passed over. There were numerous open leads in this area, and there could have been dozens or hundreds of other animals in each herd.

As there were adequate open leads in all directions, the whales were in no danger of being closed in by the ice, though the James Bay area was just experiencing the coldest weather of the winter with temperatures down to -40 F. There was probably no escape from the area as northern James Bay and Hudson Bay had long stretches of solid ice pans or land-fast ice, but the narrow tidal channels and the spacing of the islands in southern James Bay ensured open leads on at least one side of each island regardless of the directions of the winds or tidal currents.

There apparently is adequate food in the area as all the whales appeared in good condition. In comparison, white whales I observed that had been trapped in the land-fast ice near Grise Fiord during the winter of 1966–67 were in poor condition. The James Bay whales were often resting or swimming near the surface, but dove as the helicopter approached to about 100 m.

I have travelled by air over the sea ice of James Bay at various times during March and April and have always found many open leads near the islands or in the straits (Fig. 2). Even with the previous night's temperature of -37 F on April 2, the larger leads had either no new ice or only thin pans of floating new ice and slush. It seems reasonable, therefore, to assume that large numbers of white whales normally winter successfully in the persistent leads adjacent to the small islands of James Bay.

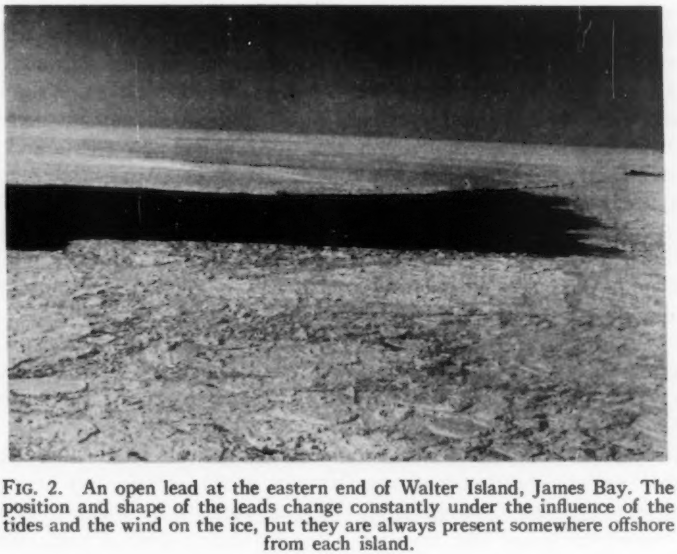

FIG. 2. An open lead at the eastern end of Walter Island, James Bay. The position and shape of the leads change constantly under the influence of the tides and the wind on the ice, but they are always present somewhere offshore from each island.

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Marine Toxins from the Pacific — Ciguatoxin: not an In Vivo Anticholinesterase[1,2]

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RAYNER, MARTIN D., MORRIS H. BASLOW, AND THOMAS I. KOSAKI. 1969. Marine toxins from the Pacific — ciguatoxin: not an in vivo anticholinesterase. J. Fish. Res. Bd. Canada 26: 2208–2210.

Although ciguatoxin, prepared from the liver and flesh of the moray eel *Gymnothorax javanicus*, is an in vitro anticholinesterase, it has not been found to be an in vivo anticholinesterase. Respiratory failure and death due to ciguatoxin is not a function of cholinesterase inhibition as previously speculated, although the toxin may have a widespread action at cholinergic junctions.

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INHIBITORY ACTION OF CIGUATOXIC extracts on the acetylcholinesterase of human and bovine red blood cells was first reported by Li (1965) from in vitro experiments in which a shark liver extract was used as the source of toxin. On the basis of in vitro assay with pure enzyme and substrate, this observation was extended to pure ciguatoxin (CT) prepared from flesh of the moray eel *Gymnothorax javanicus*, from Johnston Island by Scheuer et al. (1967). Banner (1967), using the same assay technique, showed the anticholinesterase activity of semipurified *Gymnothorax* extracts to be significantly correlated with the mouse intraperitoneal toxicity; Kosaki and Stephens (1967) showed the same with pupillary miosis in rabbits.

Although these results indicate that CT is an in vitro anticholinesterase, it is not clear that this action is a specific inhibition of acetylcholinesterase or that this represents the primary pharmacological action. Certain observations would imply a different mechanism of action. Ogura showed a noncompetitive antagonism of the neuromuscular actions of acetylcholine by methanol extracts of CT red snapper flesh (Ogura et al., 1967); there was no indication of the initial facilitatory action that would have been expected from a typical anticholinesterase. Neuromuscular blockade was also found with *Gymnothorax* extracts and this blockade was partially reversed by the anticholinesterase drug physostigmine (Kosaki and Anderson, 1968). The respiratory actions of these extracts appeared to be significantly different from those produced by typical anticholinesterase drugs (Rayner et al., 1968) and the in vitro anti-enzymic activity of semipurified CT was found to be nonspecific (Baslow

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[2] Contribution No. 334 of the Hawaii Institute of Marine Biology, University of Hawaii, Honolulu, Hawaii.

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and Rayner, 1969). Independently, Ogura and his co-workers (1968) concluded that CT is not a typical anticholinesterase since it failed to produce the EEG arousal characteristic of these agents. We now report the results of an experiment designed to measure in vivo inhibition of acetylcholinesterase.

Materials and methods — CT extracts for this study were kindly made available by Dr P. J. Scheuer and his co-workers. Two toxic extracts were used: a *Gymnothorax* liver extract purified by thin-layer chromatography and a *Gymnothorax* flesh extract purified by a silicic acid column. The highly purified liver extract was chosen since the possibility existed that the conflicting results of the earlier work were caused by impurities in the CT extracts used. This extract was lethal to 10-g mice when injected intraperitoneally at 0.03 mg/kg (average time till death was 120 min) and caused respiratory failure within 20 min when injected intravenously in rats at a dosage of 0.015 mg/kg. A semipurified *Gymnothorax* flesh toxin was also used to allow a more direct comparison with previous work on similar toxins (e.g., Banner, 1967; Kosaki and Stephens, 1967; Kosaki and Anderson, 1968; Rayner et al., 1968). The mouse intraperitoneal toxicity of this extract was 2 mg/kg; respiratory failure in intravenously injected rats occurred within 20 min at dosage levels greater than 1 mg/kg.

Female albino rats weighing 180–220 g were anesthetized with sodium pentobarbital (25 mg/kg). Respiration and common carotid pressure were monitored with a Grass Polygraph; a tracheal cannula was inserted to facilitate artificial respiration should this become necessary. A 1-ml blood sample was withdrawn from each rat before intravenous injection of the test substance. Control experiments were carried out with the following anticholinesterase drugs: physostigmine, neostigmine, tensilon, and paraoxon. A second 1-ml blood sample was taken 10–30 min after anticholinesterase injection. The same procedure was followed in CT-treated rats except that after the larger doses of toxin, which caused heart failure within 5 min of toxin injection, the second blood sample was removed immediately after heart failure.

Rat blood-cell cholinesterase was assayed by an automated *p*H stat method (Nabb and Whitfield, 1967) using 2×10^{-3} M acetylcholine perchlorate as substrate and titrating to *p*H 8.0. Such assays show a repeatability of ±10% in the experience of this laboratory.

Results — In these studies no inhibition of blood cell cholinesterase in vivo was observed, and it appears that there may actually be a small elevation in cholinesterase activity in the animals at acute stages of liver CT intoxication. Control rats treated with various anticholinesterases show the expected depression in blood cell cholinesterase activity at acute stages of intoxication (Table 1).

Discussion — The observed lack of anticholinesterase activity of CT in vivo supports previous pharmacological evidence (Rayner et al., 1968) that indicated that the respiratory failure and death produced by CT were not results of its anticholinesterase activity. If CT is not an in vivo anticholinesterase, the previous in vivo work should probably be reinterpreted to indicate a widespread action at cholinergic junctions.

Acknowledgments — The authors gratefully acknowledge the assistance of Mr W. L. Yauger, Pacific Biomedical Research Center, University of Hawaii, who carried out the cholinesterase assays.

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Table 1. The effect of ciguatoxin (CT) preparations and several anticholinesterases on rat blood-cell cholinesterase in vivo.

Treatment	Concn (*mg/kg*)	No. of animals	Effect on cholinesterase (% change)
Purified liver CT	0.015	2	5 I[a]
	0.030	2	20 I
	0.045	2	5 I
	0.050	2	5 I
Avg dose	0.035		
Avg response			9 I
Semipurified flesh CT	0.5	2	6 D
	1.0	5	4 I
	1.25	1	19 D
	1.5	4	14 D
	2.0	3	12 D
Avg dose	1.3		
Avg response			7 D
Control anticholinesterase agents			
Physostigmine	1,000	2	60 D
Neostigmine	6,000	1	93 D
Tensilon	50,000	2	42 D
Paraoxon	3,000	2	67 D

[a] I = increase; D = decrease.

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Sebastodes flavidus, a Shelf Rockfish Feeding on Mesopelagic Fauna, with Consideration of the Ecological Implications

Walter T. Pereyra,[1] William G. Pearcy,[2] and Forrest E. Carvey, Jr.[1]

Pereyra, Walter T., William G. Pearcy, and Forrest E. Carvey, Jr. 1969. *Sebastodes flavidus*, a shelf rockfish feeding on mesopelagic fauna, with consideration of the ecological implications. J. Fish. Res. Bd. Canada 26: 2211–2215.

Yellowtail rockfish (*Sebastodes flavidus*) aggregated along the southern edge of the Astoria Canyon, probably in response to the increased availability of mesopelagic food. The myctophid *Stenobrachius leucopsarus* was the dominant food organism.

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On the afternoon of June 26, 1967, a moderate scattering layer was detected on a 38.5 kHz echo sounder at depths of 60–73 fath above a bottom depth of 75 fath along the southern edge of the Astoria Canyon (Fig. 1). Subsequent sampling in this layer with a large midwater trawl resulted in a 375-lb catch that was predominantly (93%) yellowtail rockfish, *Sebastodes flavidus* Ayres.

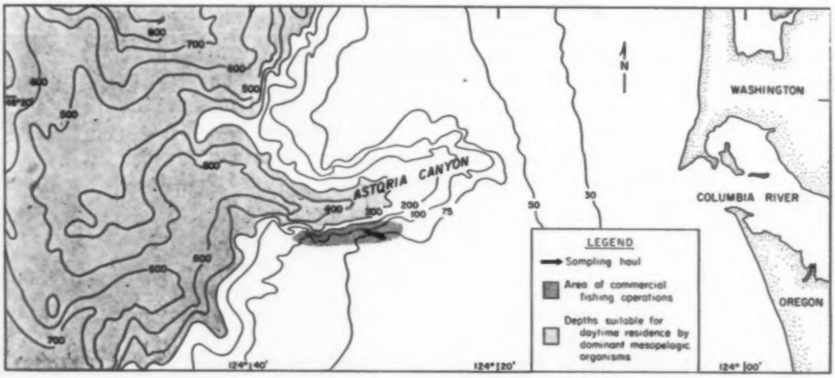
ASTORIA CANYON
WASHINGTON
COLUMBIA RIVER
OREGON
N
LEGEND
Sampling haul
Area of commercial fishing operations
Depths suitable for daytime residence by dominant mesopelagic organisms
124°40'
124°20'
124°00'
900
800
700
600
500
300
200
100
75
50
30

Fig. 1. Astoria Canyon area showing location of sampling haul, commercial fishing operations, and usual daytime depth of dominant mesopelagic organisms. All soundings are in fathoms.

The yellowtail rockfish were feeding predominantly on mesopelagic fauna. In this note we shall show that the aggregation of this species in this area was probably a response to the increased availability of the mesopelagic food.

Area — The Astoria Canyon begins about 10 miles off the mouth of the Columbia River and ranges in depth from 70 to 1000 fath. For 11 miles

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it is quite steep and from 4 to 7 miles wide. The southern wall of the Canyon adjacent to where we trawled drops 350 fath in about 1½ miles (Fig. 1).

This area is strongly influenced by the discharge from the Columbia River. When our observations were made in June, discharge from the Columbia was at its peak. Of greater importance to our discussions is the presence of a surface current flowing to the south (Budinger et al., MS, 1964).

Methods — The scattering layer was sampled with a BCF Mark I Universal trawl having a 121-ft headrope and footrope and constructed of 2½-inch mesh throughout (Jurkovich, 1968). The last 12 ft of the codend were lined with ½-inch mesh. The trawl was spread with 5- by 8-ft aluminum hydrofoil doors (McNeely et al., 1965). The trawl was positioned in the scattering layer with a strain-gauge-type, real-time depth telemetry system utilizing electromechanical trawl cables (Lusz, 1968).

Stomachs were removed from the fish a short time after capture and preserved in 10% formalin. The size and sex of all fish sampled were noted. The number of organisms and displacement volume of the stomach contents were determined on shore.

Feeding on mesopelagic fauna — The stomach contents of 22 *S. flavidus* (17 male and 5 female), ranging in size from 44 to 52 cm, were examined. Food volumes in the stomachs ranged from 11.2 to 54.1 ml (Table 1) with a trend toward higher volumes in the larger fish. None of the stomachs was empty. Also, less than 10% of the contents were unidentifiable. On the average about 90% of the stomach contents were mesopelagic organisms, with the myctophid *Stenobrachius leucopsarus* (Eigenmann and Eigenmann)[3] being found in all stomachs and accounting for 75% of the average food volume (Fig. 2). The crustaceans *Sergestes similis* Hansen, *Thysanöessa spinifera* Holmes, and *Pasiphaea pacifica* Rathbun were the only other species frequently encountered (Table 2).

TABLE 1. Range and average volume of food groups from 22 stomachs of *Sebastodes flavidus*.

Food group	Volume	
	Avg (*ml*)	Range (*ml*)
Myctophid (*Stenobrachius leucopsarus*)	21.1	7.9–45.1
Crustacea	3.8	0.2–9.8
Squid	1.0	0.0–7.1
Other	2.3	0.9–5.4
Total	28.2	11.2–54.1

Four widow rockfish, *Sebastodes entomelas* (Jordan and Gilbert), which were captured with the *S. flavidus*, had also fed on mesopelagic fauna, but

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[3] This species has been recorded as *Lampanyctus leucopsarus*; however, the subgenus *Stenobrachius* is distinctive enough to warrant full generic status (R. L. Wisner as cited by Berry and Perkins, 1966).

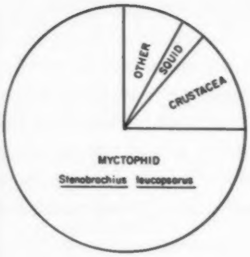

FIG. 2. Composition of stomach contents of 22 *Sebastodes flavidus*.

the percentage that *S. leucopsarus* comprised of the stomach contents was lower (54%)

While removing *S. flavidus* stomachs it was noted that, internally, the fish were very oily. This condition was not noted for specimens of this species trawled from other shelf areas off Oregon and Washington. The lipid content of *S. leucopsarus* from Oregon coastal waters is high and averages over 50% of the dry weight (Heath and W. G. Pearcy, unpublished data), suggesting that the observed oily condition of *S. flavidus* may be caused by feeding on organisms of high lipid content.

TABLE 2. Frequency of occurrence of species identified from 22 stomachs of *Sebastodes flavidus*.

Species	Frequency of occurrence
Fish	
Stenobrachius leucopsarus	1.00
Sebastodes sp.	0.05
Invertebrates	
Sergestes similis	0.91
Thysanöessa spinifera	0.86
Pasiphaea pacifica	0.55
Euphausia pacifica	0.23
Unidentified euphausiid	0.18
Hyperia hystrix	0.05
Gonatus fabricii	0.27
Gonatus sp.	0.05
Abraliopsis felis	0.05

M. S. Alton (personal communication) noted that *S. flavidus* taken incidentally with Pacific hake, *Merluccius productus* (Ayres), off Oregon and Washington fed primarily on the euphausiids *T. spinifera* and *Euphausia pacifica*, and that deeper mesopelagic organisms were not normally encountered in the stomachs examined. This feeding behavior differs from that which we observed and may be due to the uniqueness of the situation in the area of the Astoria Canyon, as will be shown.

Ecological considerations — From the limited evidence available it appears that *S. flavidus* aggregate on the south side of the Astoria Canyon in response to the availability of mesopelagic food organisms transported to that area. Aron (1962) and Pearcy (1964) found that *S. leucopsarus* was the numerically dominant species of fish in oceanic midwater trawl catches off Washington and Oregon. Pearcy and Laurs (1966) and Taylor (1968) demonstrated that this species makes extensive vertical migrations. Both of these factors may contribute to the high availability of *S. leucopsarus* as a forage organism for *S. flavidus* in the observational area.

The action of a southerly current on the vertically migrating fauna was most likely the transport mechanism bringing about the observed distributional pattern. Within 1½ miles of the site of observed concentration of *S. flavidus*, the Astoria Canyon is sufficiently deep (300 fath) to allow the mesopelagic species to descend to their usual daytime depth (Fig. 1). At night when the mesopelagic fauna ascends from the Canyon into the upper layers, the prevailing southerly currents need only carry these mid-depth organisms a short distance (1–2 miles) before they are over depths shallower than their normal daytime depth. Thus with a current of about 0.5 knots (Budinger et al., MS, 1964) and a darkness period of 5–6 hr the fauna could easily be transported to the edge of the Canyon where it would descend at dawn to the vicinity of the outer shelf. Because the depth of the shelf would be shallower than the usual daytime residence depth of the vertically migrating organisms, they would be concentrated at or near the bottom where they might be more available to predatory species such as *S. flavidus*. When feeding aggregations of *S. flavidus* occur, they in turn may be more available to fishermen. Several Soviet and United States trawlers were observed fishing intensively at depths from 65 to 80 fath along the southern edge of the Canyon. Both fishing activity and sound scatterings were absent on the north side of the Canyon. These differences support the hypothesis that vertically migrating fauna were transported to the south where predators such as *S. flavidus* were concentrated.

We surmise that *S. flavidus*, which were sampled in the afternoon (1340–1420 hr P.S.T.), were feeding intensively during daytime hours after the mesopelagic organisms had descended to the vicinity of the outer shelf. This conclusion is based on the fact that (1) none of the stomachs was empty and most were full, and (2) the stomach contents were relatively undigested.

A similar interaction between a current and a vertically migrating population, resulting in a net transport of oceanic prey into shallow water where they are vulnerable to neritic predators, was postulated by Isaacs and Schwartzlose (1965) off Baja California. Their study was based on the distribution of sound scatters and lacked direct evidence for predation provided by an examination of the stomach contents of suspected predators. Scattering layers have been observed in other areas of the North Pacific where the shelf drops rapidly into deep water (R. L. McNeely, personal communication; Donaldson, MS, 1968).

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Microsporidial Infection of English Sole, *Parophrys vetulus*

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Wellings, S. R., L. E. Ashley, and G. E. McArn. 1969. Microsporidial infection of English sole, *Parophrys vetulus*. J. Fish. Res. Bd. Canada 26: 2215–2218.

Infection of two English sole, *Parophrys vetulus*, probably by *Glugea hertwigi*, is described. Numerous *Glugea* cysts (xenomas), measuring 0.5–1.0 mm in diameter, were located throughout the entire intestine distal to the stomach and including the pyloric caecae. A few cysts were located in the mesentery, pancreas, and liver. Microscopically, the cysts were composed of a hyaline connective tissue capsule, enclosing an intermediate layer of syncytium and a central mass of mature spores.

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Microsporidians are parasitic sporozoans known to infect a variety of fishes, including *Gasterosteus aculeatus* (Putz, 1964), *Osmerus mordax* (Linton, 1901; Mavor, 1915; Schrader, 1921; Haley, 1952), *Pleuronectes platessa* (Johnstone, 1901), *Fundulus heteroclitus* (Bond, 1937, 1938), *Salmo gairdneri* (Wales and Wolf, 1955), *Cottus* sp. (Wales and Wolf, 1955), and *Notemigonus crysoleucas* (Summerfelt, 1964).

Infection of the pleuronectid *Pleuronectes platessa* (Johnstone, 1901) by *Glugea stephani* has previously been indicated. The purpose of the present report is to describe the pathology of infection of English sole by a *Glugea*, probably *Glugea hertwigi* Wissenberg, 1911. This is the first reported occurrence of *Glugea* infection in this species of flatfish.

Materials and methods — Two specimens of English sole, *Parophrys vetulus*, were collected on October 10, 1967, in Yaquina Bay, Oregon, by Mr Wally DeBen of the Federal Water Pollution Control Administration. The fish were fixed in 5–10% formalin, photographed, and dissected. Specimens for light microscopy were embedded in paraffin, sectioned at 8 μ, stained with hematoxylin and eosin, mounted on glass slides, and photographed by means of a Zeiss photomicroscope.

Gross pathology — The two diseased fish measured 11.7 and 12.1 cm in total length. In each instance one translucent ring was observed on the auditory ossicles, indicating that the fish were in the 2nd year of life.

The visceral mass of both specimens was grossly enlarged, causing the abdominal wall on both the eyed and blind sides to bulge markedly (Fig. 1). In the larger specimen the terminal bowel was prolapsed so that the everted mucosa protruded approximately 1 cm beyond the normal location of the vent (Fig. 1). The wall of the prolapsed bowel was filled with numerous white, opaque, spherical cysts clearly visible at the mucosal surface and measuring about 0.5–1 mm in diameter.

The entire gut wall distal to the stomach and including the pyloric caecae was massively thickened because of numerous 0.5- to 1-mm cysts located at all levels in the wall from the mucosa to the serosa (Fig. 2). The white opaque cysts were identical to those of the prolapsed bowel described above. A few similar cysts were located in the mesentery, pancreas, and liver, but were not observed elsewhere.

Histopathology — The cysts (Fig. 3, 4), located in connective tissue of the gut wall, liver, pancreas, or mesentery, were enclosed in a capsule, which appeared to be composed of hyalinized connective tissue. A layer of loose host connective tissue, infiltrated by lymphocytes, *surrounded* the capsule. A thin syncytial layer of cytoplasm containing large vesicular host nuclei together with developmental stages of the microsporidians was closely applied to the *inner surface* of the hyaline capsule (Fig. 5). The great bulk of each cyst consisted of a huge central mass of mature spores (Fig. 6) measuring 4–4.5 μ long. Each spore was oval in shape and enclosed in a capsule consisting of a transparent refractile material resembling chitin. Internally, the spores contained a vacuole at one pole and a "polar filament" at the other. The dimensions, shape, and internal morphology of the spores were consistent with *G. hertwigi.*[1]

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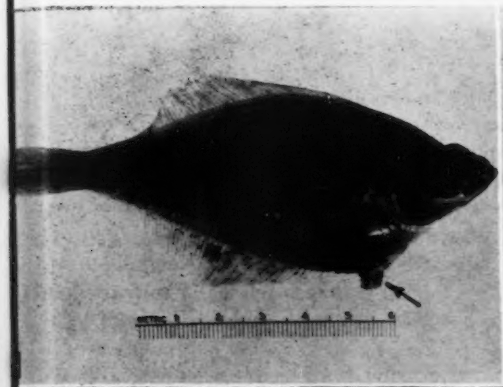
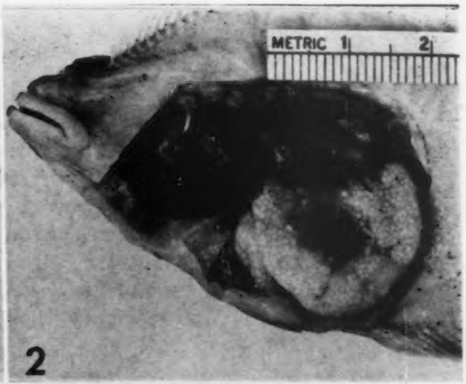
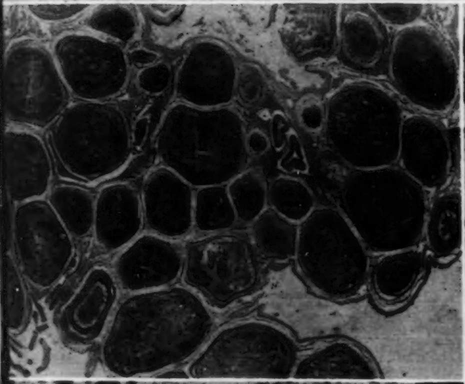
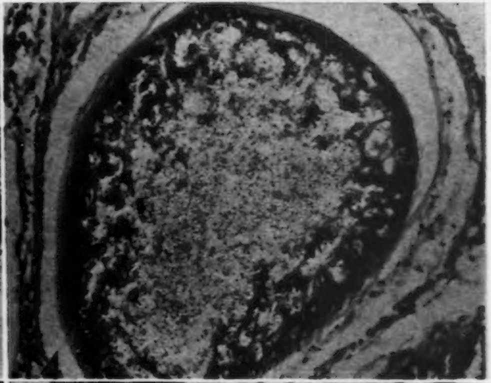
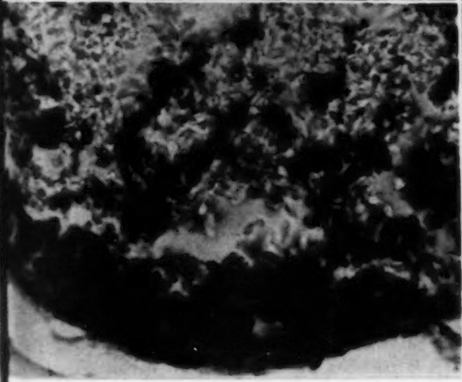
[1] The authors are grateful to Dr Glen Hoffman of the Eastern Fish Disease Laboratory at Leetown, West Virginia, and to Dr R. Weissenberg for helping tentatively identify the spores.

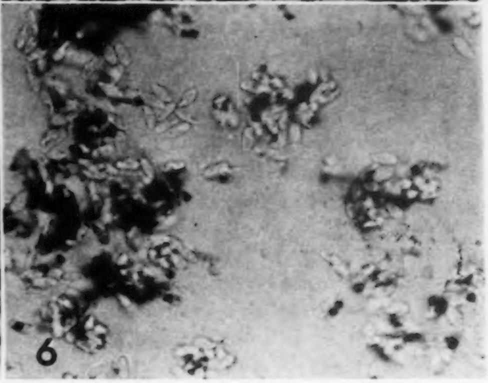












1. English sole, *Parophrys vetulus*, with swelling of abdomen. Prolapsed rectum with minute, 0.5–1 mm diam, *Glugea* cysts, visible as numerous highlights (arrow) in the photograph.

2. Dissection of abdomen of English sole, showing numerous parasitic cysts, causing massive thickening of the intestinal wall, and responsible for abdominal enlargement. A cyst in the liver is indicated by an arrow.

3. Photomicrograph illustrating numerous parasitic cysts replacing the structure of the intestinal wall. Remnants estinal epithelium are observed in the intestinal lumen at the lower left; the peritoneal surface is at the bottom and also to the left. Hematoxylin and eosin stain. 16.5×.

4. Parasitic cyst illustrating the surrounding fibrous encapsulation of host origin. Hematoxylin and eosin. 225×.

5. Higher magnification of wall of cyst with hyaline capsule (C), protoplasmic layer (P), and spores (S). 900×.

FIG. 6. Spores of *Glugea* inside the cysts. 900×.

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Discussion — Microsporidians of the genus *Glugea* are among the smallest sporozoan parasites that infect fish. Typically, development occurs in host cytoplasm, leading to massive cellular hypertrophy, characterized by nuclear division without cytokinesis. Thus, a greatly hypertrophied, multinucleated cell is produced. The parasites multiply and develop by schizogony and sporogony, yielding mature spores, which accumulate in a central vacuole. Eventually the spherical host cells attain a diameter of 0.5–1.0 mm. At this time they consist of a huge central mass of mature spores, surrounded by a thin rim of multinucleated host cytoplasm, and are called "Glugea cysts," or "xenomas" (Weissenberg, 1949).

In the course of natural infections the cysts rupture, releasing the spores into the water, where, apparently, they are ingested by the next host. The spores attach to the intestinal epithelial cells, perhaps by means of the polar filament, which is released and uncoils under the influence of host digestive enzymes. The sporoplasm then leaves the spore case as an amoeboid cell (amoebula), which penetrates the mucosa, and enters a host cell to complete the cycle. Cellular infection may occur locally or after hematogenous systemic or regional dissemination. At this time the parasitic cell is termed a trophozoite, and it further develops by the process of schizogony and sporogony, characteristic of many parasitic sporozoans (Arme and Owen, 1964; Fantham et al., 1941; Doflein, 1898, 1916; Haley, 1952; Hoffman, 1967; Jakowska and Nigrelli, 1959; Johnstone, 1901; Kudo, 1924; LeGault and Delisle, 1967; Mavor, 1915; Putz, 1964; Reichenbach-Klinke and Elkan, 1965; Schrader, 1921; Stunkard and Lux, 1965; Weissenberg, 1911a, b).

Microsporidial infection of the digestive tract, similar to that described in the present report, was described in winter flounders, *Pseudopleuronectes americanus,* by Stunkard and Lux (1965).

There are several previously published examples of infection of pleuronectids by species of the genus *Glugea.* Johnstone (1901) indicated that *G. stephani* was a common parasite of flatfishes. Fantham et al. (1941) observed a microsporidian, thought to be *G. stephani,* in the winter flounder, *P. americanus,* and in *Limanda ferruginea,* caught off the eastern coast of North America near Halifax.

Several reports indicate that smelt, *Osmerus eperlanus,* are unusually susceptible to *Glugea* infection (Mavor, 1915; Schrader, 1921; LeGault and Delisle, 1967). Schrader's (1921) work describes the intestine as the primary seat of infection with some cysts in other viscera. The cysts were up to 3 mm in diameter, white, and located in the mucosa in early infections. As the cysts enlarged they apparently pushed their way through the muscular coat of the intestine to lie just beneath the serosal surface. The entire length of the intestine distal to the stomach was involved, and some cysts were also found in the gonads and liver. This pathology is strikingly similar to that described in the present work.

Recently, Weissenberg (1968) described similar infection of *G. aculeatus* by *Glugea anomala.* The disease was transmitted by feeding spores to young larvae. Migratory phagocytic mesenchymal cells of the midgut were parasitized and developed into the typical *Glugea* cysts. Weissenberg gives detailed descriptions of the intracellular development of the parasite.

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Stomach Contents of Juvenile Pacific Salmon in Chatham Sound and Adjacent Waters

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Manzer, J. I. 1969. Stomach contents of juvenile Pacific salmon in Chatham Sound and adjacent waters. J. Fish. Res. Bd. Canada 26: 2219–2223.

Stomach contents of young Pacific salmon in Chatham Sound and adjacent waters of northern British Columbia from June through August indicated interspecific differences in the kinds of organisms consumed. Pinks (*Oncorhynchus gorbuscha*) and chums (*O. keta*) were mainly planktophagous, copepods and Larvacea (*Oikopleura* spp.) being most important; cohos (*O. kisutch*) were piscivorous, herring larvae (*Clupea* spp.) and sand lance (*Ammodytidae* spp.) being important; sockeye (*O. nerka*) were mainly planktophagous but fish also were important. With pinks and chums, while they were still relatively abundant along the beaches, the dominant food item progressively changed from copepods in the southern areas to Larvacea in the northern areas.

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In Chatham Sound and adjacent waters of northern coastal British Columbia, young Pacific salmon, especially pinks (*Oncorhynchus gorbuscha*) and chums (*O. keta*), are found in varying numbers along the beaches until early summer when they begin to move offshore (Manzer, 1956). This paper describes the diet of young pink, chum, sockeye (*O. nerka*), and coho (*O. kisutch*) salmon during residence in this region from early June to late August in 1955, but mainly during the first half of this period.

Methods — The study was incidental to studies conducted on the distribution and migration of young salmon in Chatham Sound and adjacent waters. Beach sampling locations (Fig. 1) were established in early June on the basis of fishability and were revisited sequentially four times at approximately 2-week intervals. About mid-July when young salmon became scarce along the beaches, fishing operations shifted "offshore" into deeper water.

Young salmon in littoral areas were caught in beach seines (approximately 37 m long, 1.8 m deep), whereas those in deeper water were taken in purse seines (approximately 110 m long, 5.4 m deep). Each type of gear was constructed from 1.9-cm mesh (stretched measure) cotton webbing. Time spent fishing in a particular location ranged from 1½ to 2½ hr. On occasion more than one catch was made, in which case these were combined.

Samples of each species were labelled according to place, date, and time of capture, wrapped in cheese cloth, and preserved in wooden tubs containing 10% formalin. Most fish were sufficiently small that their stomach contents were preserved simply by immersing the fish in the preservative. With relatively large fish, usually cohos, an incision was made in the abdominal wall so that the preservative could enter the body cavity.

In this study, "stomach" is considered to be that portion of the alimentary tract between the oesophagus and intestine. The stomach contents of each fish were identified in the laboratory according to broad taxonomic groups (mainly order) with the aid of a binocular microscope. Contents rendered unrecognizable either through mastication or digestion were classed as "unidentifiable." Stomach parasites were not considered as part of the stomach contents.

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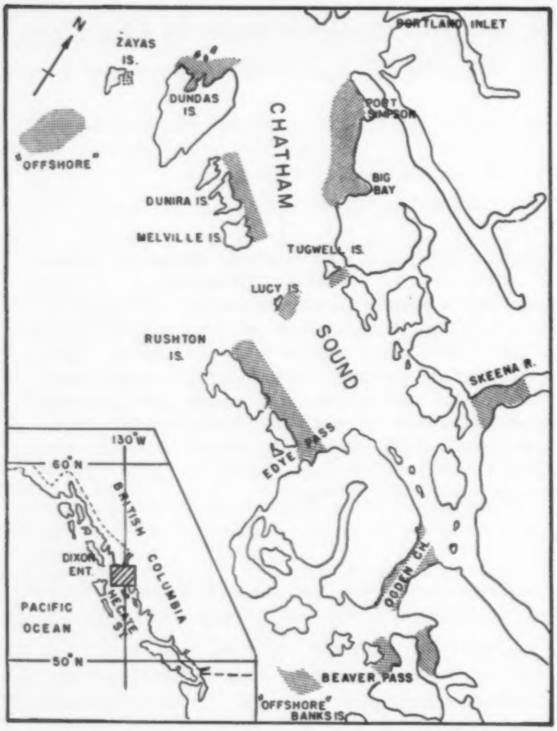
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BEAVER PASS
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BANKS IS

FIG. 1. Chatham Sound in northern British Columbia and sampling localities (shaded areas).

Although fish were not aged, length data recorded at the time of stomach examination indicate that all fish were in their 1st year of ocean life.

The importance of each taxonomic group in a given species' diet was determined from the percentage of stomachs examined that contained organisms of the group (occurrence method) and from the percentage that each kind of organism formed of the total volume of stomach contents of the fish in the sample (volumetric method). Regarding the latter method, initially the percentage for each group was determined from volumetric data based on water displacement. As proficiency in estimating volumes was achieved, volumes of sparsely represented groups were subsequently estimated by eye. The relative merits of using these methods in food studies of fishes have been discussed by Hynes (1950) and Windell (1968).

Results and discussion — For each species a very high proportion of the stomachs examined contained food. The food spectra of pinks, chums, sockeyes, and cohos for the entire study period were reasonably similar, but the degree of importance of each taxonomic group varied (Table 1). In general, pinks and chums were planktophagous, copepods and Larvacea (*Oikopleura* spp.) being most important. Cohos were mainly piscivorous, herring larvae (*Clupea* spp.) and sandlance (*Ammodytidae* spp.) occurring most frequently. Sockeyes overlapped these two groups somewhat in their diet, being mainly planktonic feeders but fish were also important.

TABLE 1. Relative importance of various taxonomic groups consumed by juvenile salmon in Chatham Sound and adjacent waters, early June to late August 1955. F = % occurrence; V = % of volume; + = <0.5%.

Taxonomic group	Pink		Chum		Sockeye		Coho	
	F	V	F	V	F	V	F	V
Chaetognatha	+	+	+	+	–	–	1	+
Cladocera	–	–	–	–	–	–	1	+
Gastropoda	2	+	+	+	2	+	–	–
Polychaeta	–	–	1	+	–	–	–	–
Ostracoda	2	+	3	+	1	1	–	–
Copepoda	48	31	29	19	45	48	7	6
Cirripedia	13	6	5	2	3	1	–	–
Mysidacea	+	+	1	+	–	–	1	+
Cumacea	1	+	2	+	1	+	–	–
Isopoda	1	+	1	+	–	–	1	+
Amphipoda	7	1	8	2	3	3	11	3
Euphausiacea	7	3	7	4	9	1	4	3
Decapoda	14	4	20	5	7	5	14	8
Crustacean remains	–	–	–	–	–	–	4	1
Insecta	8	1	23	5	10	4	19	5
Larvacea	41	40	62	51	25	14	1	+
Fish	2	5	5	4	19	24	53	70
Eggs	1	+	+	+	–	–	–	–
Unidentifiable	18	7	13	4	5	2	8	2
No. stomachs examined	537		410		98		144	
% with food	95		95		91		87	
No. fish measured	470		329		50		144	
Size range of fish measured (*mm*)	35–114		32–106		57–122		62–147	

Examination of the stomach contents of pinks and chums taken in different areas during three sampling circuits from June 8 to July 18, while juveniles were eadily available along the beaches, revealed that for each species the primary food item changed progressively from copepods in the southern areas to Larvacea in the northern areas (Table 2). Since this spatial trend was observed for both pinks and chums, it seems reasonable to think that the phenomenon was the result of differences in the relative contribution of the two kinds of organisms to the local food resource.

The kinds of organisms found in the stomachs of young salmon during this study in general were similar to those reported by earlier investigators working in widely separated areas (Chamberlain, 1907; Fraser, 1946; Foskett, 1951; Synkova, 1951). One outstanding difference, however, is the hitherto unreported large incidence of Larvacea in the diets of pinks and chums.

TABLE 2. North–south changes in relative importance of copepods and Larvacea in the diet of underyearling pink and chum salmon in Chatham Sound and adjacent waters, June 8–July 18, 1955. For each species, only areas are considered that yielded samples in each of the three sampling circuits during this period. See Table 1 for legend. North is Dundas–Zayas Island.

Area	Pinks					Chums				
	No. stomachs examined	Copepods		Larvacea		No. stomachs examined	Copepods		Larvacea	
		F	V	F	V		F	V	F	V
Dundas–Zayas Is.	105	34	20	67	61	42	7	>1	76	84
Port Simpson–Big Bay	–	–	–	–	–	68	6	3	75	67
Melville–Dunira Is.	71	32	22	54	54	41	12	8	71	62
Lucy Is.	–	–	–	–	–	28	18	6	89	76
Edye Pass–Rushton Is.	140	71	64	19	9	111	42	26	60	40
Ogden Ch.–Beaver Pass	46	87	76	13	6	77	64	44	39	28

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Effect of the Rate of Storm Growth on Subsequent Surge Elevations

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HOLLAND, GEOFFREY L. 1969. Effect of the rate of storm growth on subsequent surge elevations. J. Fish. Res. Bd. Canada 26: 2223–2227.

The surge elevation due to an instantaneously applied generating force over one-half of an infinite rotating sea was given by Crease (Proc. Roy. Soc. London, Ser. A: 233, 1955). This note describes the effect of modifying the applied force to allow it to reach its maximum value over a finite period of time.

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THE SOLUTION FOR the elevation due to the instantaneous application of a given force over one-half of an infinite rotating sea was given by Crease (1955). The mathematical model used was idealistic and several assumptions were made to obtain an analytical solution. However, even simplified models can give useful indications of the dynamics that exist in actual physical systems, and some of the physical characteristics of the surge can be revealed by an investigation of the changes in the analytical solution due to basic modifications in the given conditions. This note describes an interesting result found when the applied force was allowed to grow from zero to its final value over a finite period of time rather than being instantaneously applied at its maximum value. It is shown that the maximum elevation attained by a surge will be lower, and the time taken to achieve this maximum longer, for those storms having the slower rate of growth. Both of these characteristics have been observed in nature. Researches into the correlation between unpredicted sea levels (residuals) at Liverpool and the neighbouring barometric distribution led to a large measure of success when the distribution did not change too rapidly (Proudman and Doodson, 1929). However, a study of the effect of storms seemed to show that when the distribution changed suddenly the effects on the sea level followed very rapidly and reached magnitudes much greater than those produced by more slowly changing disturbances.

It can be accepted, therefore, that for a calculation of the worst conditions for coastal flooding the instantaneous force could be used. The predicted time taken to reach the maximum from the initial conditions, however, will be too short, and as the surge elevation must be superimposed on the tide this discrepancy must not be overlooked. For the purposes of design the maximum predicted surge could be superimposed on the highest high water. The following mathematics demonstrate that a rapidly intensifying storm of short duration can generate more severe surges than those due to a storm of possibly greater magnitude and persistence that achieves its full force in a gradual manner. This knowledge will be useful to research workers engaged in hindcasting and forecasting techniques of storm surge generation.

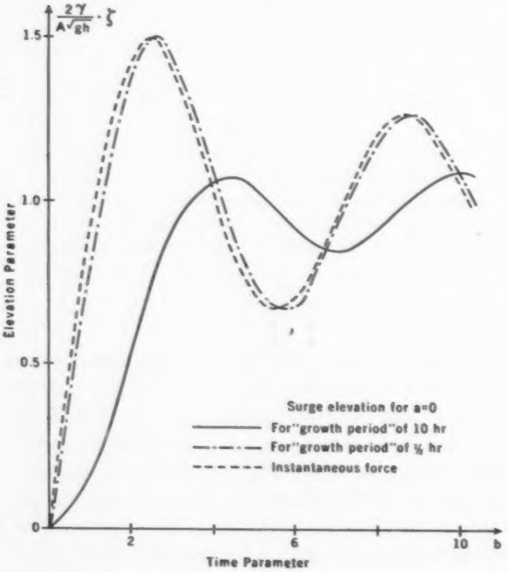

FIG. 1. Comparison of elevation produced for various growth periods.

The equations of motion relating to an infinite rotating sea of constant depth are as follows:

$$\frac{\delta U}{\delta t} - \gamma V = - g \frac{\delta \zeta}{\delta x} - \frac{1}{p} \frac{\delta P_a}{\delta x} + \frac{1}{ph} \tau_{xs} \qquad (1)$$

$$\frac{\delta V}{\delta t} + \gamma U = 0 \qquad (2)$$

and the continuity equation is

$$\frac{\delta U}{\delta x} = - \frac{1}{h} \frac{\delta \zeta}{\delta t} \qquad (3).$$

In these equations, nonlinear terms have been omitted on an order-of-magnitude consideration (Charnock and Crease, 1957). Conditions have been assumed constant in the y direction and the equations averaged throughout the depth h. In the equations the depth-averaged velocities are U and V in the x and y directions, respectively, the elevation of the free surface ζ, the atmospheric pressure P_a, the stress on the surface in the x direction τ_{xs}, and the coriolis parameter γ. Bottom friction, earth curvature, and the variation of latitude have been neglected as being small.

By eliminating the velocities from these equations and taking the applied force as

$$E(x,t) = \frac{1}{pg} \left(\frac{\delta P_a}{\delta x} - \frac{\tau_{xs}}{h} \right), \qquad (4)$$

the equation for ζ is found to be

$$\left[\frac{\delta^2}{\delta x^2} - \frac{1}{gh} \left(\frac{\delta^2}{\delta t^2} + \gamma^2 \right) \right] \zeta = - \frac{\delta E}{\delta x}, \qquad (5)$$

the integration constant being zero if at some time ζ, $\frac{\delta^2 \zeta}{\delta t^2}$, and $\frac{\delta E}{\delta x}$, are all zero.

Equation 5 can be solved by a Green function (Crease, 1955) or by a Laplace transform (Holland, MS, 1969) to give the general solution

$$\zeta = \frac{\sqrt{gh}}{2} \int_0^t dt_0 \int_{-\infty}^{\infty} \frac{\delta E}{\delta x_0} (x_0,t_0) \cdot J_0 \left[\gamma \left\{ (t-t_0)^2 - \frac{(x-x_0)^2}{gh} \right\}^{\frac{1}{2}} \right] . \; H \left[(t-t_0) - \frac{|x-x_0|}{\sqrt{gh}} \right] dx_0 , \qquad (6)$$

J_0 being a Bessel function of the first kind.

The step function H found in the integrand dictates that there can be no elevation at a point outside the generating area until enough time has elapsed for the surge to reach that point from the edge of the generating area travelling at the maximum group velocity of $\sqrt{gh}$.

If we now assume a force instantaneously created at time $t = 0$ over the semi-infinite range $-\infty < x < 0$ we can represent this force as

$$E(x,t) = -A \cdot H(-x) \cdot H(t), \tag{7}$$

A being an arbitrary constant.

The force represents a wind or pressure system or a combination of both, and by substitution into equation 6

$$\zeta = \frac{A\sqrt{gh}}{2} \int_0^t J_0\left[\gamma\left\{(t-t_0)^2 - \frac{x^2}{gh}\right\}^{\frac{1}{2}}\right] \cdot H\left[(t-t_0) - \frac{|x|}{\sqrt{gh}}\right] dt_0 \tag{8}$$

then, using the nondimensional parameters

$$\left.\begin{array}{llll} t - t_0 = \tau & & \gamma\tau = \beta & \\ \gamma t = b & & \dfrac{\gamma x}{\sqrt{gh}} = a, & \end{array}\right\} \tag{9}$$

the solution can be simplified to

$$\frac{\gamma\zeta}{\sqrt{gh}} = \frac{A}{2} \int_{|a|}^{b} J_0\left[(\beta^2 - a^2)^{\frac{1}{2}}\right] d\beta \tag{10}.$$

In nature, however, it would be expected that a force would build up to its maximum value over a finite period of time. Therefore, the generating force could be modified to be

$$E(x,t) = -A \cdot H(-x) \cdot H(t) \cdot \tanh(\epsilon_0 \gamma t) \,. \tag{11}.$$

This force starts from zero and increases asymptotically to its maximum value at a rate governed by the parameter ϵ_0. We can give an indication of the rate of growth by taking that time during which the storm achieves a certain percentage of its final value. As $\tanh \theta = 0.9$ when $\theta = 1.5$, the force will achieve 90% of its final value at the time t given by $\gamma t = 1.5/\epsilon_0$. We could call this period the growth period $T = 1.5/\epsilon_0\gamma$.

The new solution for the elevation ζ' is found to be

$$\frac{\gamma\zeta'}{\sqrt{gh}} = \frac{A}{2}\int_{|a|}^{b} \tanh \left\{ \epsilon_0(b-\beta) \right\} \cdot J_0\left[(\beta^2-a^2)^{\frac{1}{2}} \right] d\beta \qquad (12).$$

The solution ζ differs from ζ' only by the inclusion of a factor tanh $\epsilon_0(b-\beta)$ in the integrand of the latter. The two solutions can be evaluated numerically and plotted as functions of nondimensional times or distances. In the figure this has been done for the simple case $a = 0$, which corresponds to the edge of the generating area $x = 0$. Growth periods corresponding to times of ½ hr and 10 hr for latitudes such as the Labrador coast (55°) and slightly greater, about 0.6 hr and 12 hr for the coast of Nova Scotia (45°), have been plotted. For a given value of ϵ_0 the periods will vary inversely as γ ($=2\Omega\sin\varphi$). It can be seen from these curves that the maximum elevation decreases and the time taken to achieve this maximum increases as the period of growth increases. Finally it should be stated that this note deals with only one characteristic of storm surge phenomena; others, such as the effect of the velocity of the disturbance, its strength, size, and the geographical features of the area concerned all have to be considered. Several such modifications to the given force have been investigated (Holland, MS, 1969).

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Simple Stream Tank Simulating a Rapids Environment

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GEE, J. H., AND V. G. BARTNIK. 1969. Simple stream tank simulating a rapids environment. J. Fish. Res. Bd. Canada 26: 2227–2230.

The construction of an inexpensive stream tank providing a rapids environment of 0.6 m^2 with a maximum velocity of 0.5 m/sec is described. Compressed air and a large aquarium are required to create an adequate volume of flow.

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CREATING A LOTIC environment in the laboratory to study stream-dwelling organisms is often a costly and difficult procedure. The greatest problem to overcome is rapid movement of a large volume of water. For example, a flow of 0.61 m/sec (2 ft/sec) at 15.2 cm (6 inch) depth over a 0.61 m width requires a volume of flow of 0.057 m^3/sec (12.5 imp. gal/sec). The purpose of this note is to describe a simple and inexpensive method of creating a small rapids area of 0.55 m^2 (6 ft^2) in the laboratory.

Artificial streams may be classified into either open or closed systems. In the former, water runs into and out of the stream tank; in the latter, water is continuously recirculated.

An open system requires large volumes of uncontaminated water, usually from a nearby stream. Problems associated with this system include (*a*) difficulty in controlling turbidity and temperature of water, and (*b*) food for fish washing out before it is eaten. This system imposes relatively few restrictions on the area of stream created and is most suitable for large fishes. An open system was used and described by Lindroth (1954) and Fabricius and Lindroth (1954).

Smaller fishes are held more conveniently in closed systems and the above problems are reduced, although the area of stream created is usually smaller. In such a system, water must be replaced occasionally to avoid contamination. It is usually circulated by one or more pumps either on a horizontal plane (Northcote, 1962; Raleigh, 1967) or on a vertical plane (Hartman, 1965a, b). Techniques for studying aquatic insect larvae in simulated lotic environments have been reviewed by Craig (1966).

Our stream tank is a closed system. Water circulation is based on a method used by Gee (1968) where bubbling-air diffusor stones on the bottom at one end of an aquarium create a circular current of water on a vertical plane. Compressed air and a large aquarium are required.

The aquarium used (1.22 × 0.61 × 0.61 m) was constructed of angle-iron (3.6 × 3.6 cm) with 0.9-cm plate-glass sides and ends and a 1.2-cm plate-glass bottom. Dow Silicone Aquarium Cement was used with a 0.3-cm layer of cement between the glass and the frame. An additional layer of cement was applied from the inside, once the glass was in place, to all seams along the bottom and ends. All exposed portions of the frame were coated with Tremclad Rust Preventative Paint.

Compressed air (12–15 psi) was available at four outlets, each of which was capable of providing 0.65 liters/sec of air to air diffusor stones in water 0.61 m deep. Each of these outlets supplied air through plastic tubing connected to three 30.5-cm long air diffusor stones, which were located on the bottom at one end of the aquarium. Rising bubbles of air from the stones lifted water at that end of the aquarium and caused a current of water to be carried along the surface, down the opposite end, and along the bottom, thus causing circulation on a vertical plane.

We utilized only that area of current at the surface of the aquarium. A false bottom of Plexiglas (88.9 × 59.1 × .64 cm) was suspended across the width of the aquarium (Fig. 1). It came within 19 cm of one end of the aquarium and 11.5 cm of the other. Rising air bubbles and water passed up through the largest opening, over and along the false bottom, and down through the smaller opening. Fish were retained over the false bottom by stainless steel screens (1.6 meshes/cm, 0.12-cm diam) running across the width of the aquarium at either end of the false bottom.

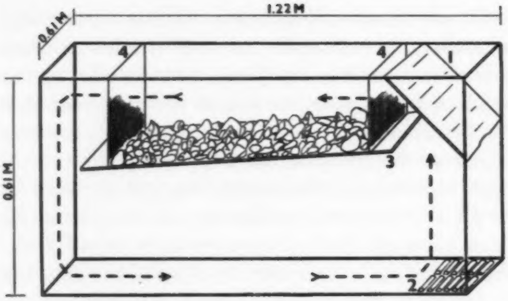

FIG. 1. Diagram of stream tank. The arrows indicate the direction of flow. 1 = deflector plate; 2 = air diffusor stones; 3 = false bottom; 4 = screen. The heating element, supporting wires for the false bottom, and plastic tubing supplying compressed air to the diffusor stones have been omitted.

A glass deflector plate (59.1 × 27.9 cm) was placed at an angle of 45° over the air diffusor stones to direct the rising flow of water over the false bottom. It was held against the sides and end of the aquarium with silicone aquarium cement. A small opening in the center of its lower edge allowed passage of plastic tubing from the compressed air outlets to the air diffusor stones.

The false bottom was strengthened by four strips of Plexiglas (59.1 × 2.54 × 1.3 cm) cemented across the top of the sheet. These supports were placed 22.9 cm apart and came within 3.1 cm of either end. Holes of 0.32-cm diam were located 0.63 cm from the ends of these supports. The false bottom was suspended by stainless steel wires (0.12-cm diam). They were tied through these holes and attached to clips at the top of the aquarium frame. The false bottom, capable of supporting gravel to a depth of 10 cm had a rise of 1 cm for every 12 cm of its length. This allowed maximum volume of flow, which was 0.014, 0.020, 0.022, or 0.026 m^3/sec (0.50, 0.70, 0.76, or 0.91 ft^3/sec) with 1, 2, 3, or 4 outlets of compressed air open. The maximum velocity attained over the false bottom was 0.50 m/sec with four outlets open.

The aquarium was kept in a cold (9 C) constant environment room. Water temperature was controlled by a 0.91-m length of chromalox tubular heating element (1000 w) located between the deflector plate and the upstream screen. It was capable of raising the temperature 1.7 degrees C/hr and was controlled by a thermostat and a time clock. Thus the heater could be operated over a particular period each day to result in a daily rise and fall in temperature similar to that of natural streams.

"Day" illumination was provided by two lights, each connected to a time clock. A 60-w bulb was held in a lamp above the aquarium and light was directed upwards to reflect off a white ceiling onto the stream tank. This source of illumination was provided over the complete length of day. A 150-w spotlight, directed onto the stream tank was used to provide bright midday illumination over part of the day. This pattern of illumination approximated that received in nature.

In this stream tank, food items such as frozen brine shrimp, dried flake, or small euchytraeid worms can be recirculated over the false bottom and are constantly available to fish.

One could not expect to create a velocity of water much faster than 0.61 m/sec in such a stream tank. The volume of water circulated depends on the supply of compressed air and the depth at which air diffusor stones are located. Depths less than 30 cm produce little circulation. It would be possible to increase the area of stream conditions without increasing the volume of water circulating by making the aquarium longer. The cost of such a stream tank is minimal. By installing glass in the aquarium ourselves, we kept the total cost to approximately $365.00.

At present we are using two of these stream tanks to investigate and compare the prespawning and spawning behaviour of two species of dace (Cyprinidae: *Rhinichthys*). These fishes readily establish and defend up to five territories at any one time in such a stream tank and we have recorded several successful spawning acts. Other aspects of behaviour of small stream-dwelling fishes could also be studied. In addition, such stream tanks could be easily modified to conduct experiments on substrate or current preference.

Thus it is possible to create on a limited scale and expense, a rapids environment similar to that found in streams. Such a stream tank permits observations that are often most difficult to make in nature because of the shallow, turbulent, fast-flowing water.

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Preliminary Diagnoses of New Septibranch Species from the Eastern Pacific (Bivalvia, Anomalodesmata)

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BERNARD, F. R. 1969. Preliminary diagnoses of new septibranch species from the eastern Pacific (Bivalvia, Anomalodesmata). J. Fish. Res. Bd. Canada 26: 2230–2234.

Preliminary diagnoses of six western American septibranch bivalves are given to avoid manuscript names being used for an extended period. A revision of the recent representatives of the order in the eastern Pacific has been submitted to the Allan Hancock Foundation, University of Southern California, for publication.

Received January 24, 1969

A REVISION OF the eastern Pacific recent Septibranchida based upon the extensive Allan Hancock Foundation materials, and the collections of the Fisheries Research Board of Canada, has been submitted to the Foundation. As publication will be delayed it is considered undesirable to allow new names to remain in manuscript form. With permission of the Allan Hancock Foundation, descriptions of the new species are given here. Systematic lists, anatomical descriptions, and extended comparative discussions are omitted, as they will appear in the complete revision at a later date.

Pelseneer (1888) proposed the division Septibranchia in the Anatinacea. As no standardized endings exist for the ordinal categories, Septibranchida is used following Keen (1963) rather than the cumbersome Septibranchoidea put forward by a number of workers, notably Vokes (1967).

Revision has shown that the Septibranchida is a polyphyletic order conveniently divided into two superfamilies, the Poromyacea and the Verticordiacea; the former is an early offshoot of the protobranchian phylogeny and the latter a comparatively recent adaptation of the anomalodesmacean stock to a carnivorous existence. Eight genera and 47 species are present in the eastern Pacific. Most are small, obscure mud dwellers of deep and abyssal waters that demonstrate peculiar modifications. In all species the ctenidia are reduced or lost, in some instances being replaced by a muscular septum. The digestive system is characteristically modified for dealing with large food particles, the entire order being carnivorous upon small invertebrates, notably crustaceans and annelids.

Class	Bivalvia
Subclass	Anomalodesmata
Order	Septibranchida
Superfamily	Poromyacea Dall 1886
Family	Cuspidariidae Dall 1886
Genus	*Cardiomya* A. Adams 1864

Cardiomya isolirata sp.n.
(Fig. 1)

Diagnosis — Shell small, slightly inequivalve, inequilateral, anterior evenly rounded. Rostrum short, abruptly truncate, distally gaping, dorsal margin concave, ventral margin arcuate. Disc ornamented with 12–20 rounded uniform ribs, which extend to include ventral portion of rostrum. Beaks inflated. Periostracum cinerous and caducous. Interior shiny, ventral margin slightly crenulated by overlying ribs. Length 7.9; height, 4.8; width, 3.7 mm.

Holotype — Deposited in the Allan Hancock Foundation. Pelecypoda No. 163.

Type locality — 32°32′16″N, 117°15′28″W. Hancock Station 1241-41-D2 off Point Loma, California, in 33 fath, February 23, 1941.

Range — 34.1°N–27.8°N. 55–183 m. Off Los Angeles County, California, to Baja California, Mexico.

The species has been confused with *Cardiomya balboae* Dall as both bear approximately the same number of ribs. *Cardiomya isolirata* is a much heavier shell with a denticulated margin. The disc is uniformly ribbed, as is the anterior portion of the short arcuate rostrum. The inside surface of *C. balboae* is smooth, whereas that of *C. isolirata* is crenulated by the overlying ribs.

Genus *Cuspidaria* Nardo 1840

Cuspidaria parapodema sp.n.

(Fig. 2)

Diagnosis — Shell inflated, fragile, translucent, inequilateral, subequivalve. Peristracum very thin, often with adherent heavy desquamating arenaceous coating. Beaks inflated, hinge long. Anterior rounded, posteriorly merging with rostrum. Exterior smooth with few incremental lines extending into rostrum. Interior highly polished. Length, 15.2; height, 9.1; width, 7.2 mm.

Holotype — Deposited in the Alan Hancock Foundation. Pelecypoda No. 162.

Type locality — 33°49'10"N, 118°26'10"W. Hancock Station 1132-40 off Redondo Beach, California, in 43 fath, May 5, 1940.

Range — 34.4°N–24.9°N. 53–275 m. Off Los Angeles County, California, to Baja California, Mexico.

This allopatric species is closely related to *Cuspidaria apodema* Dall. *Cuspidaria apodema* is a benthal species whereas *C. parapodema* is part of the Californian shallow water fauna. In *C. apodema* the lateral tooth is short, involuted, and subtriangular, whereas in *C. parapodema* it is curved and elongated. It is the most abundant of the Californian cusparids.

Genus *Poromya* Forbes 1844
Subgenus *Dermatomya* Dall 1889

Poromya (Dermatomya) canadensis sp.n.

(Fig. 4)

Diagnosis — Shell iridescent, translucent, small and thin; equivalve, inequilateral. Surface smooth with shiny, strongly adherent periostracum. Posterior subtruncate, anterior rounded. Hinge weak, dentition obsolete, with small chondrophore. Beaks prominent, not inflated. Ligament weak and short. Interior shiny white, pallial and muscular scars slight. Length, 6; height, 4.1; width, 3.9 mm.

Holotype — Deposited in the National Museum of Canada, Ottawa, Mollusc type Collection Catalogue No. 46460.

Type locality — 48°02'00"N, 125°45'10"W. Fisheries Research Board of Canada Station No. 63-835, off Vancouver Island, British Columbia, in 534 fath, September 6, 1964.

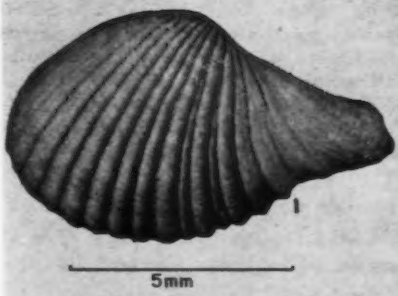
Range — West coast of Vancouver Island.

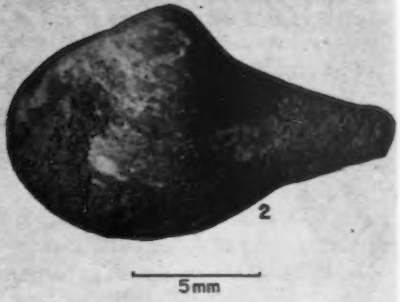
The species superficially resembles the outline of *Poromya* (*Cetoconcha*) *smithi* Dall; however, the shell surface is smooth, the shell is proportionately thicker, and the beaks are more prominent.

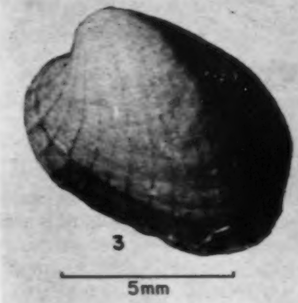
Superfamily Verticordiacea
Family Verticordiidae Stolicza 1871
Genus *Lyonsiella* Friele 1879

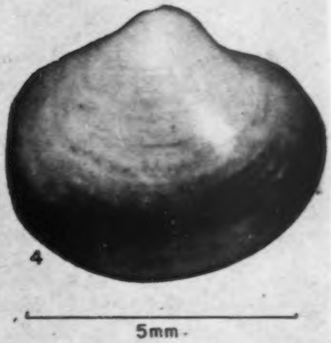
Lyonsiella quaylei sp.n.

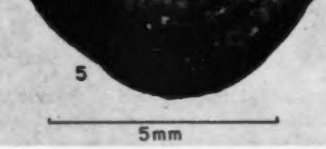
(Fig. 3)











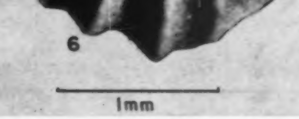


FIG. 1. ***Cardiomya isolirata*** sp.n., holotype.

FIG. 2. ***Cuspidaria parapodema*** sp.n., holotype.

FIG. 3. ***Lyonsiella quaylei*** sp.n., holotype.

FIG. 4. ***Poromya (Dermatomya) canadensis*** sp.n., holotype.

FIG. 5. ***Verticordia (Haliris) spinosa*** sp.n., holotype.

FIG. 6. ***Verticordia (Trigonulina) hancocki*** sp.n., holotype.

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Diagnosis — Shell small, thin, inflated, subquadrate, inequilateral, inequivalve. Periostracum strongly adherent, silky, cinerous, overlying 20 slight rays. Entire surface covered with microscopic granules, enlarged and radially arranged on the rays. Lunule impressed. Beaks small. Margin straight, sharp. Interior pearly. Hinge weak with internal ligament, large cylindrical lithodesma. Left valve with small shelf below umbo. Length, 8.1; height, 6.8; width, 6 mm.

Holotype — Deposited in the Allan Hancock Foundation. Pelecypoda No. 164.

Type locality — 33°20′01″N, 118°13′58″W. Hancock Station 2641-54 off Catalina Island, California, in 363 fath, April 25, 1954.

Range — 47.7°N–33.3°N. Vancouver Island, Canada, to Catalina Island, California.

The species bears some resemblance to *Lyonsiella magnifica* Dall, but the shell is markedly fragile and the hinge weaker. The species has subsequently been collected off Vancouver Island and is named in honour of Dr D. B. Quayle.

Genus *Verticordia* Wood 1844

Subgenus *Haliris* Dall 1886

Verticordia* (*Haliris*) *spinosa sp.n.

(Fig. 5)

Diagnosis — Shell inflated, thin, inequilateral, equivalve. Beaks prominent. Surface ornamented with 25–30 rounded ribs that denticulate the ventral margin. Lunule impressed. Shell surface uniformly granular, becoming spinose on ventral portions of ribs. Interior smooth and shiny with slight channels corresponding to external ribbing. Right valve with lateral tooth, left valve with peglike cardinal tooth and wide bifurcated lateral tooth just below umbo. Length, 7.1; height, 6.5; width, 6.0 mm.

Holotype — Deposited in the Allan Hancock Foundation. Pelecypoda No. 166.

Type locality — 23°38′12″N, 109°30′00″W. Hancock Station 1037-40-D1 off Baja California, Mexico, in 150 fath, January 21, 1940.

Range — Type locality only.

The species bears a resemblance to *Verticordia fischeriana* Dall, but the thinner shell, more pronounced ribbing, and stronger hinge differentiate the new species.

Subgenus *Trigonulina* Orbigny 1845

Verticordia* (*Trigonulina*) *hancocki sp.n.

(Fig. 6)

Diagnosis — Shell small, inequilateral, equivalve, subquadrate, compressed. Surface sculptured with pronounced costae that denticulate the ventral margin. Entire surface covered with exiguous granules, foveolated between costae. Lunule deep. Interior brilliantly nacreous, crenulated by overlying costae. Adductor scars faint. Hinge heavy, massive cardinal tooth in right valve. Length, 2.1; height, 1.4; width, 1.1 mm.

Holotype — Deposited in the Allan Hancock Foundation. Pelecypoda No. 165.

Type locality — 3°00′22″N, 78°19′47″W. Hancock Station 587 off Gorgona Island, Colombia, in 40–60 fath, February 24, 1938.

Range — Type locality only.

The species is much more inequilateral than either of the other two Eastern Pacific species, *Verticordia aequacostata* Howard 1950 and *Verticordia ornata* (Orbigny 1853).

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Color in Raw and Cooked Atlantic Salmon (*Salmo salar*)

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SAITO, A. 1969. Color in raw and cooked Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 26: 2234–2236.

Carotenoid contents in raw and cooked Atlantic salmon, and in canned Pacific salmons, were compared. The color, as measured by the Gardner color difference meter, was found to be correlated with the chromaticity coordinate $x\%$, or with Hunter's value $+a$. The color of Atlantic salmon flesh, compared with that of Pacific salmon flesh (canned), is significantly redder than chum and pink salmon, but less red than coho or sockeye in terms of the carotenoid content, $x\%$ or $+a$ values, and visual color judgment.

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THE CAROTENOIDS of salmon have been studied by many investigators since Bailey (1937) suggested the presence of two carotenoid pigments in the sockeye. Kanemitsu and Aoe (1958a, b) identified astaxanthin, its mono-cis isomer, and astacene and its corresponding isomer, in five species of North Pacific salmon. Kanemitsu and Matsuura (1961) investigated the change in the pigment content, calculated as astaxanthin, during the storage of canned red salmon. Schmidt and Idler (1958) used a Gardner automatic color-difference meter for assessment of the color of sockeye meat before and after canning. The equipment they used gave color values as *Rd*, *a*, and *b* in the Hunter color system that could be converted to the tristimulus values of the C.I.E. (Commission Internationale d'Éclairage) scale (Hunter, 1948). Based on their results Cuthbert (1968) devised a colorimeter for the in-cannery color sorting, and the Hunter *a* color score, used as an objective measure of the cooked or canned color of Pacific salmon flesh, correlated satisfactorily with the visual assessment of the product. This investigation consisted of comparison of the

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color of the Atlantic salmon with that of common Pacific species, and observation of the relationships between the carotenoid content and the color measured by reflectance.

Materials and methods — A Gardner automatic colorimeter, model AC-3 (Gardner Lab., Inc., Maryland) was used for the measurement of the color. This instrument provides directly the tristimulus values X, Y, and Z in the C.I.E. system.

Four different species of canned Pacific salmon, chum (*Oncorhynchus keta*), pink (*Oncorhynchus gorbuscha*), sockeye (*Oncorhynchus nerka*), and coho (*Oncorhynchus kisutch*), sold under a total of six different brand names, were purchased at a local market. The opened cans were placed upside down on a funnel covered with cheesecloth and drained for 5 min. This separated the orange-colored oil, and other liquid from the flesh. The skin, flesh, and bones were separated from each other with a spatula, and the flesh was flaked by a metal spoon for color measurement. Two Atlantic salmon (*Salmo salar*) (♂, 65 cm, 3375 g; ♀, 67 cm, 3600 g) were caught at Chester Bay, N.S., in May 1967, and another two (♂, 52 cm, 1697 g; ♀, 57 cm, 1654 g) were caught at West River, Sheet Harbour, N.S., in September 1967. Each fresh fish was cut into four portions (head, back, belly, and tail), the skin and bones were removed from a portion, and the flesh was chopped into paste in a blender. The flaking method used on cooked products was not applicable to the raw product. For cooking, a portion of the raw Atlantic salmon was cut from the caudal center portion and heated in steam for 15 min under 15 lb/in^2 pressure. Then the cooked flesh was treated as canned flesh.

The crude lipid extracted by Bligh and Dyer's method (1959) was dissolved in acetone and the absorption spectrum was determined on a Unicam auto-recording spectrophotometer, Model-800, between 360 and 600 mμ. Total carotenoids including astaxanthin, astacene, and unidentified isomers were calculated as astaxanthin ($E^{1\%}_{1\,\mathrm{cm}}$ 472 mμ = 2200 in acetone).

To measure the color of the sample, flaked flesh was compressed into a transparent polystyrene petri dish (internal diam. 2.0 inches; Ames Lab-Tek Inc., Illinois), and color was measured through the bottom of the dish on a Gardner colorimeter with an aperture diameter of $1\frac{3}{8}$ inches. The instrument was adjusted to give the reading of the reference standard, white plate GC-6619 (Gardner Lab. Inc.), before each sample was measured. The reflectance scale X, Y, and Z readings were taken as the mean value of triplicate readings in three different positions. The chromaticity coordinates $x\%$ and $y\%$, and Hunter's chromaticity value $+a$ (redness) and $+b$ (yellowness) were calculated by formula (table for conversion, Gardner Lab. Inc.).

Results — The results of the analyses of the raw, cooked, and canned salmon samples are presented in Table 1.

In the raw Atlantic salmon, there was not much variation in carotenoid content or reflected color in different parts of the body. In the samples analyzed, the carotenoid content was not directly related to the lipid content of the flesh.

The cooked Atlantic salmon had a higher carotenoid content than the canned chum and pink salmon from the Pacific but a significantly lower content than the sockeye and coho. The chromaticity $x\%$ in the C.I.E. system was found to be directly related to the carotenoid content in the cooked fish. The Y or the $y\%$ showed no definite relationship to the carotenoid content. The Hunter $+a$ value was also proportional to the carotenoid content in confirmation of observations previously reported (Schmidt and Idler, 1958). The $+a$ value more forcefully accentuates the color differences between samples as compared with the chromaticity $x\%$. Using the observed a values, it can be concluded that the color of the Atlantic salmon is significantly redder

TABLE 1. Lipids, carotenoids, and color values of raw and cooked Atlantic salmon and canned Pacific salmon.

Species (*no. of samples*)	Crude lipids (*g/100 g dry wt*)	Carotenoids (*mg/100 g dry wt*)	Tristimulus value Y	Chromaticity coordinate $x\%$	Hunter's chromaticity value $+a$
Raw Atlantic					
Back (5)	16.8	1.44±0.37	0.264	41.0	16.98
Head (3)	23.7	1.51±0.73	0.230	41.0	18.00
Belly (3)	44.0	1.19±0.31	0.232	42.1	18.23
Tail (3)	11.3	1.55±0.72	0.250	41.8	17.80
Cooked Pacific					
Chum (9)	12.3	0.91±0.02	0.384	37.5	5.49±1.55
Pink (5)	22.0	1.01±0.02	0.378	38.0	6.96±1.50
Atlantic (4)	25.0	1.34±0.03	0.439	38.7	9.44±1.63
Coho (20)	20.7	3.71±0.05	0.329	41.4	14.29±2.47
Sockeye (6)	25.7	4.15±0.81	0.268	44.0	17.45±3.60

than the chum and pink but less red than the coho and sockeye. These color relationships were also noted by visual comparison. It is recognized that the individual differences caused by environment, seasonal variation, the variables of processing conditions, and storage have not been investigated here.

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Effect of Magnesium Sulphate and Cryoprotective Agents on Free Fatty Acid Formation in Cold-Stored Fish Muscle

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Rainbow trout (*Salmo gairdneri*) muscle was stored in 5% $MgSO_4$ in Ringer's solution, 15% dimethyl sulphoxide in Ringer's solution, and 6% dextran in 0.9% NaCl, at −10 C for periods of 2, 4, 8, and 12 weeks. The regular increase in free fatty acid (FFA) content was used as an index of muscle degradation. For the 12-week storage period at −10 C, the FFA content varied from 17.9 μmoles in fresh muscle to a maximum of 222 μmoles/100 g muscle for nonimmersed muscle. Samples immersed in $MgSO_4$ solution alone showed a consistent statistically significant inhibition in FFA formation when compared with immersed and nonimmersed muscle controls. Immersion in DMSO produced the least visual deterioration in appearance, colour, and firmess of texture.

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THE POSSIBILITY that $MgSO_4$ retards the development of toughness in cold-stored fish muscle when injected into fish antemortem was explored by Buttkus and Tarr (1962). Tomlinson et al. (1965) showed that there is an uptake of Mg^{2+} ion in fish stored in refrigerated sea water. Whether the Mg^{2+} ion taken up by the fish muscle in this fashion exerts any effect on the frozen storage characteristics of the muscle is unknown. The present report concerns the effect of $MgSO_4$ on the formation of free fatty acids (FFA) in frozen fish muscle. The influence of certain known cryoprotectors, dimethyl sulphoxide (DMSO) and dextran, on the degradation of lipids was also examined. Since deterioration of cold-stored fish muscle can be accompanied by the breakdown of phospholipids by phospholipases with the release of FFA (Dyer and Fraser, 1959; Olley and Lovern, 1960; Bligh, 1961; Jonas and Tomlinson, 1962), the concentration of FFA found was used as a measure of muscle degradation.

Two-year-old rainbow trout (*Salmo gairdneri*) were stunned, decapitated, and skin-free fillets were prepared and stored at −10 C as follows. One set of fillets was wrapped in Saran Wrap and used as nonimmersed control. Another set was immersed in Ringer's solution (Hawk et al., 1949) and used as immersed control. The test storage solutions were 5% $MgSO_4$ in Ringer's solution, 6% dextran in 0.9% NaCl, and 15% DMSO in Ringer's solution (Rowe, 1966; Karow et al., 1967). One pair of fillets weighing 200 g was completely immersed in each solution (300 ml) contained in a wide-mouthed plastic jar (diam 2.5 inches, height 6.5 inches). The solutions were cooled to 0 C before introduction of the fillets. The jars were kept at −10 C and samples were removed at intervals of 2, 4, 8, and 12 weeks and thawed in water at 37 C. Thawed muscle (80 g) was cut up into small pieces and homogenized in a Waring Blendor, and the total fat was extracted by the method of Bligh and Dyer (1959). Phospholipids were removed from the chloroform layer by silicic acid chromatography (Olley and Lovern, 1960) and the FFA in the eluate titrated with 0.5 N alcoholic KOH with phenolphthalein as indicator.

With all the treatments used, there was a progressive increase in the FFA concentration in the cold-stored muscle throughout the 12-week period (Table 1). The FFA concentration rose from 17.9 μmoles in fresh muscle to 222 μmoles/100 g in the nonimmersed control protected with Saran Wrap. A statistical comparison of the differences between the treatments is shown in Table 2. A consistently significant difference was not observed in FFA formation in samples immersed in dextran and DMSO solutions as compared with the immersed control. However, for samples immersed in $MgSO_4$ solution, a highly significant depressing effect on FFA release was evident after the 8th and 12th week of storage, when compared with the immersed control samples; a lower, consistently significant, concentration of FFA was maintained throughout the duration of the experiment when compared with the non-

TABLE 1. Effect of various treatments on free fatty acid content (μmole/100 g muscle) of rainbow trout muscle stored at −10 C.

Treatment	Duration of storage (*weeks*)			
	2	4	8	12
Saran Wrap	93.50±9.19[a]	155.00±7.07	188.50±10.61	222.50±2.12
Ringer's solution	85.50±6.36	100.50±21.92	177.50±9.19	223.00±14.14
Dextran (*6%*)	73.50±6.39	126.50±3.54	154.00±1.41	240.50±19.09
DMSO (*15%*)	80.50±3.54	139.00±15.56	179.00±2.83	226.00±22.63
$MgSO_4$ (*5%*)	66.50±21.92	75.50±19.09	136.00±8.49	185.00±7.07

[a] Mean value and standard deviation from two experiments. The fresh muscle contained 17.90 ± 4.24 μmoles free fatty acid per 100 g.

TABLE 2. Comparison of differences between treatments.

Treatments compared	Duration of storage (*weeks*)			
	2	4	8	12
Ringer's solution Saran Wrap	N.S.[a]	$P<0.05$	N.S.	N.S.
Ringer's solution Dextram (*6%*)	N.S.	N.S.	$P<0.05$	N.S.
Ringer's solution DMSO (*15%*)	N.S.	N.S.	N.S.	N.S.
Ringer's solution $MgSO_4$ (*5%*)	N.S.	N.S.	$P<0.02$	$P<0.05$
Saran Wrap $MgSO_4$ (*5%*)	$P<0.05$	$P=0.01$	$P=0.01$	$P<0.005$

[a] N.S. indicates a statistically nonsignificant difference. The probability level of $P \leqslant 0.05$ indicates a statistically significant difference. Calculated by application of *t*-test to the data from Table 1.

immersed control samples. Although ionic solutions in general are considered poor cryoprotectors against cell injury in freezing and thawing, $MgSO_4$ solution has inhibitory effects on the enzymic breakdown of muscle lipid components. The class of lipids that is subjected to the hydrolytic process forming FFA in fish muscle during cold storage is not known. Olley et al. (1962) found that FFA released during cold storage of cod, lemon sole, and halibut were remarkably similar, rising to a plateau of approximately 300 mg/100 g muscle with evidence showing that in these species most of the FFA arose through hydrolysis of phospholipids. Recent evidence indicates that FFA production during cold storage may also take place in some species through the hydrolysis of triglycerides (Awad et al., 1969; Bosund and Ganrot, 1969). Though the contribution of triglycerides to the formation of FFA is not established in trout muscle, the formation of glycerylphosphorylcholine and choline is evidence of the hydrolysis of phospholipids in cold-stored rainbow trout muscle (Jonas and Bilinski, 1967). However, the degree of hydrolysis appears to be more moderate in rainbow trout than in species like cod at comparable temperatures (Olley and Lovern, 1960). The FFA formed in the control samples of the trout muscle in the present experiment amounted to 220 μmoles/100 g or 66 mg/100 g (300 being used as the mean molecular weight of the fatty acids). Based on the phospholipid content of trout muscle, which is approximately 600 mg/100 g or 800 μmoles P/100 g (Gray and MacFarlane, 1961; Jonas and Bilinski, 1967), the FFA released during cold storage account for the complete hydrolysis of 14% of the phospholipid present, and they represent a smaller fraction of the total lipid, which amounted to approximately 2.3% of the muscle.

Muscle fillets immersed in DMSO solution showed very little visual deterioration in colour or firmness and were very much closer in appearance to the nonimmersed control as compared with the white, swollen, soft texture of the samples immersed in the other three solutions. Muscle immersed in dextran was slightly firmer than that in Ringer's solution and $MgSO_4$ solution. Though the use of DMSO in food is not permitted by Food and Drug Regulations, its application in research as a cryoprotector could be useful where fish is used as an experimental animal.

Most experiments with cryoprotective agents have overlooked the aspect of tissue deterioration caused by lipid breakdown because of the short duration of storage and the very low temperatures used in many instances. This experiment dealt with only two of the known cryoprotective agents; it would be interesting to explore the possibilities of other reagents, particularly in their application to fish in cold storage with and without salts.

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Otter Surface Sampler: a New Neuston Net[1]

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A new surface sampler for the collection of zooplankton and ichthyoplankton is described. The advantages it has over other neuston samplers are that it can be towed at speeds up to 11 knots, can be used in choppy waters, is easily handled by one man, has no structures preceding the mouth of the net, and is easy and inexpensive to construct.

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A SURFACE SAMPLER designed to capture small forms of zooplankton and large active juvenile fish should be capable of being towed at speeds in excess of 7 knots; few juvenile cod, capelin, herring, and silver hake have been captured in neuston nets towed below this speed. There should be no structures preceding the mouth of the net, either in or above the water, in such a way that a shadow that might warn organisms that the net is approaching is cast in the path of the net. The sampler should not run in the wake of the ship, but in undisturbed water. It should be light enough for one man to handle and not require a great deal of storage space aboard ship. The sampler should be easy and inexpensive to construct.

Before 1969, the only surface samplers that had most of these qualities were the nets described by David (1965) and the Booby II sampler described by Bieri and Newbury (1966). David's sampler was built and used extensively in a regular sampling program in St. Margaret's Bay, N.S. The Booby II was not built, for it was stated to have a top towing speed of only 3 knots. David's net was found to be too heavy and awkward to handle and store. It was felt that the skis in front of the net opening were scaring organisms and providing them with a chance to escape the net. To overcome these difficulties, the otter surface sampler, having all the desirable qualities mentioned above, was designed and built (Fig. 1, 2).

The design of the net was based on the principle of the otter boards of a bottom trawl. Attaching the towing lines a short distance back from the leading edge of one of the sides caused the net to run out and away from the ship. The angle between the tow rope and the path of the ship could be increased by attaching the tow lines further back from the leading edge.

Two models of the sampler were built and used. The first was made of $\frac{3}{4}$-inch fir plywood. This model was very rugged, but was considered to be too heavy (25 kg) to be easily handled by one man. A second sampler was constructed out of $\frac{1}{8}$-inch aluminum sheeting. This model was very durable and light (12 kg). It was the easiest to construct; a sheet of aluminum of the desired length and width was bent at a series of right angles to form the body of the sampler. The top and bottom fins were bolted to steel rods by u-bolts and set at the required angles by pushing latches attached to the fins through the proper holes in the sides of the body. The net (Fig. 3) was secured through holes in the body by a $\frac{1}{4}$-inch rope. The nylon net represented about 75% of the total cost of the sampler. The only disadvantage of the aluminum model was that it had to be provided with some form of floatation to prevent its sinking in the water when it was not being towed. A block of Styrofoam was attached to the top of the sampler for floatation.

An otter sampler fitted with a $308\text{-}\mu$ mesh net was used in a regular sampling program in St. Margaret's Bay during 1968. It was normally towed at a speed of 8 knots (Fig. 4), but was towed from a stern trawler at 11 knots and performed well. The depth to which the sampler penetrated the water depended on the relative angles of the top and bottom fins, and the speed of the boat. The relative lengths of the bridles were critical in keeping the sampler running level in the water. Normally the top bridle had to be slightly longer than the bottom one, but this depended on the height of the boat and the length of the towing rope. The lengths of the bridles were easily changed by use of the turnbuckles.

Two types of bridles were used, those with turnbuckles (Fig. 1), and those with a single wire with both ends attached to the sampler (Fig. 4). The relative lengths of the top and bottom parts of the wire were changed by pulling the wire through a wire clamp and tightening the clamp. The turnbuckle bridles were easier to adjust.

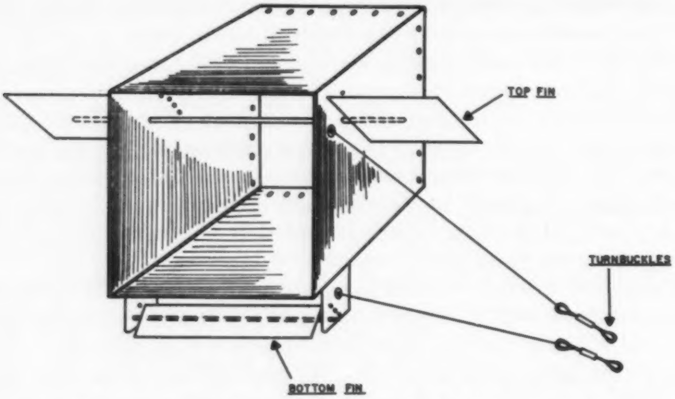
TOP FIN
TURNBUCKLES
BOTTOM FIN

FIG. 1. Perspective drawing of the body of the sampler.

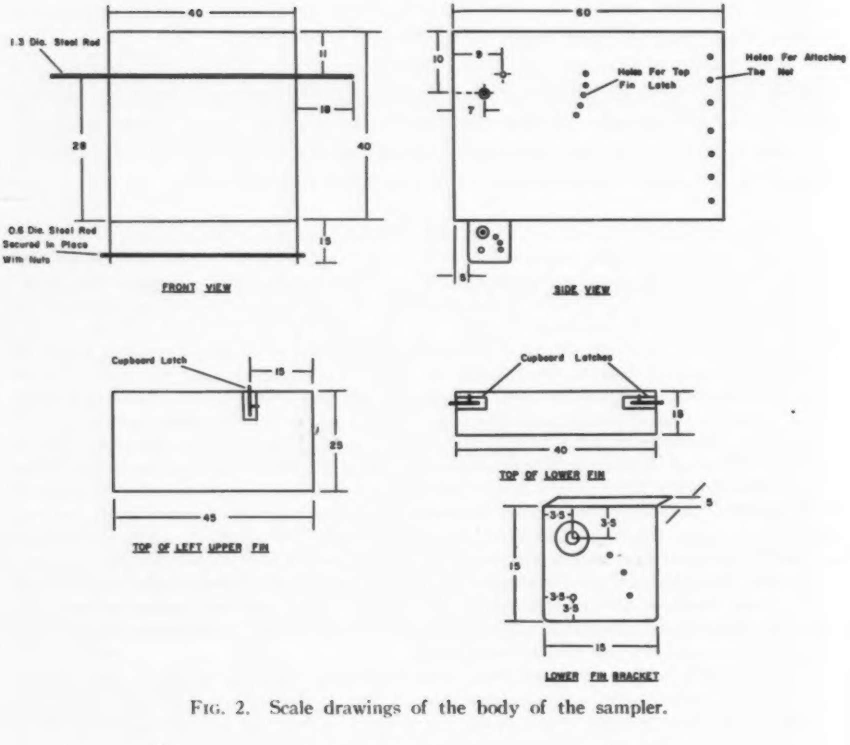
40
1.3 Dia. Steel Rod
11
18
28
40
0.6 Dia. Steel Rod Secured In Place With Nuts
15
FRONT VIEW
60
10
9
7
Holes For Top Fin Latch
Holes For Attaching The Net
5
SIDE VIEW
Cupboard Latch
15
25
45
TOP OF LEFT UPPER FIN
Cupboard Latches
18
40
TOP OF LOWER FIN
5
3·5
3·5
15
3·5
3·5
15
LOWER FIN BRACKET

FIG. 2. Scale drawings of the body of the sampler.

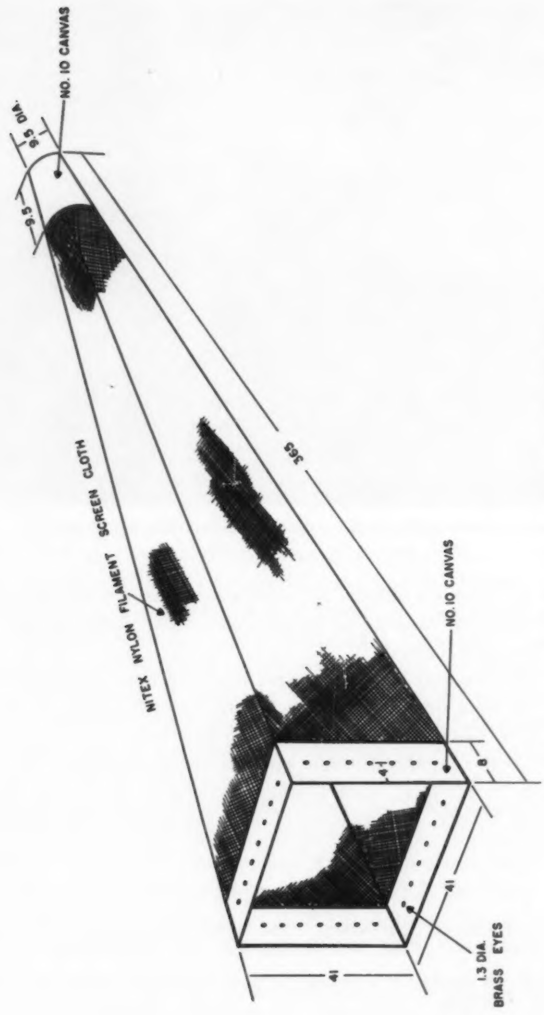
NO. 10 CANVAS
9.5 DIA.
9.5
365
NITEX NYLON FILAMENT SCREEN CLOTH
NO. 10 CANVAS
8
41
41
41
1.3 DIA. BRASS EYES

FIG. 3. Scale drawing of the net of the sampler.

The main advantage the otter surface sampler had over the neuston net described by David (1965) was that it could be used in choppy water. The sampler usually needed some adjustment when the towing direction was changed from heading into a swell to running with the swell, for it tended to surfboard down the wave. This was overcome by adjusting the bottom fin angle and the speed of the boat.

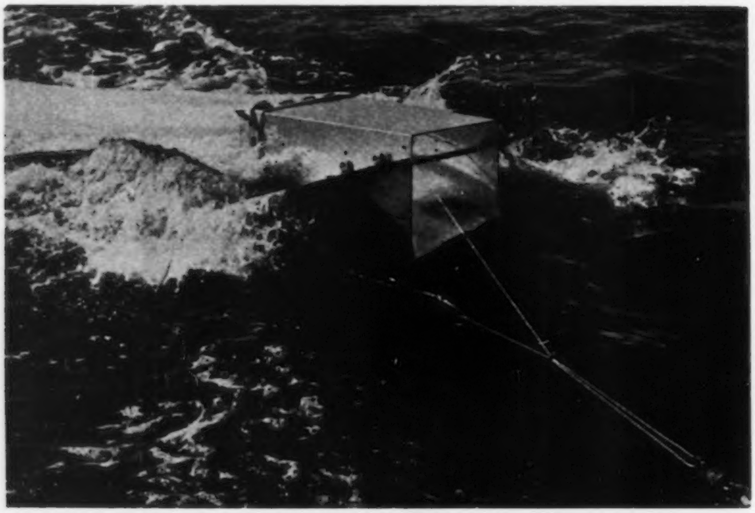

FIG. 4. Aluminium model of the otter surface sampler being towed at 8 knots.

There was no way to obtain an accurate value for the amount of water filtered, but an estimate was made by observing the depth of the sampler and knowing the distance towed.

The invertebrates and the larval and juvenile fish captured were in excellent condition. It was not uncommon to find about 50% of the larval and juvenile fish alive when taken out of the bucket.

The largest fish captured were specimens of squirrel hake, *Urophycis chuss* (Walbaum), 5 cm long, and a single specimen of the northern pipefish, *Syngnathus fuscus* Storer, 11 cm long. These fish were alive when taken from the bucket.

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Preliminary Report on the Fecundity of *Mytilicola orientalis*[1]

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JOHNSON, EUGENE A., AND KENNETH K. CHEW. 1969. Preliminary report on the fecundity of *Mytilicola orientalis*. J. Fish. Res. Bd. Canada 26: 2245–2246.

Egg cases of the endoparasitic copepod *Mytilicola orientalis* Mori were examined from samples dissected from Pacific oysters (*Crassostrea gigas*) and bay mussels (*Mytilus edulis*) from Humboldt Bay, California, Yaquina Bay, Oregon, and Willapa Bay and Hood Canal, Washington. There was little variation between the number of eggs in both egg cases from an individual female. On the bases of two egg sacs per female approximately 252–368 eggs are produced.

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MYTILICOLA ORIENTALIS is an endoparasitic copepod found in the intestinal tract of molluscs. Its presence along the Pacific Coast has been known for many years. It was originally described from Pacific oysters (*Crassostrea gigas* Thunberg) and a mussel (*Mytilus crassitesta* Lischke) in Japan. Odlaug (1946) noted the presence of the copepod in oysters (*C. gigas* and *Ostrea lurida*), bay mussels (*Mytilus edulis*), clams (*Venerupis staminea*), and eastern cup (*Crepidula fornicata*) in Washington. More recently, Chew et al. (1964) discovered the same species of parasite in *Mytilus californianus*.

Studies by the College of Fisheries during the past 5 years have elucidated some of the biological and pathological problems associated with this parasite in bivalves (Chew et al., 1965; Katkansky et al., 1967; and Sparks, 1962). To further understand the biology of *M. orientalis*, it was decided to investigate the fecundity of this species and to compare it with the egg potential of the European species *Mytilicola intestinalis*. *Mytilicola intestinalis* has been the parasite responsible for serious condition and mortality problems of bay mussels in the early 1950's and is known to develop around 200–300 eggs of fairly large size (Korringa, 1968).

Egg cases of *M. orientalis* were examined from bimonthly samples of *C. gigas* and *M. edulis* taken in Humboldt Bay, California, Yaquina Bay, Oregon, and Willapa Bay and Hood Canal, Washington. The egg cases were dissected from the female, dyed in a strong methylene blue solution (which provided a contrasting background), dissected longitudinally using a 52S cataract blade and counted under 3× magnification.

There was very little variation between the number of eggs per egg case in both egg cases from an individual female. Sixty-six pairs of egg cases were

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[1] Contribution No. 308 from the College of Fisheries, University of Washington, Seattle, Wash.

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counted with the per cent variation ranging from 5 to 22%, with only five pairs of egg cases having a variation greater than 10%. The average variation was 6.3%.

Table 1 gives the range and mean number of eggs per egg case found for *M. orientalis* dissected from *M. edulis* and *C. gigas* for Humboldt Bay, Yaquina Bay, Willapa Bay, and Hood Canal. As shown in this table, the range in the number of eggs per egg case is quite wide. Those with eggs in the lower range may not have finished egg laying when sampled. However, when considering the mean or average number of eggs per egg case, well over 100 is noted for all areas in both species of bivalves. On the basis of two egg sacs per individual female we can double these figures to get an approximate estimate of egg production. Thus, we would come up with a figure of approximately 252–368 eggs per individual female copepod as compared with 200–300 eggs mentioned above for *M. intestinalis* (Korringa, 1968).

TABLE 1. Number of egg cases counted, range in number of eggs, and average number of eggs per egg case of female *Mytilicola orientalis* dissected from the intestinal tract of *Mytilus edulis* and *Crassostrea gigas*.

Host species	Area	No. egg cases counted	Range in no. of eggs per egg case	Avg no. of eggs per egg case
Mytilus edulis	Humboldt Bay, Calif.	27	50–224	138
	Yaquina Bay, Ore.	18	33–288	154
	Willapa Bay, Wash.	38	56–310	174
	Hood Canal, Wash.	33	90–267	173
Crassostrea gigas	Humboldt Bay, Calif.	51	92–264	173
	Yaquina Bay, Ore.	33	56–330	184
	Willapa Bay, Wash.	38	66–383	161
	Hood Canal, Wash.	27	58–245	126

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Morphological Characteristics of Infectious Pancreatic Necrosis Virus in Trout Pancreatic Tissue

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Lightner, Donald, and George Post. 1969. Morphological characteristics of infectious pancreatic necrosis virus in trout pancreatic tissue. J. Fish. Res. Bd. Canada 26: 2247–2250.

Electron microscopical observations were made of infectious pancreatic necrosis (IPN) virus in thin sections of pancreatic acinar cells of rainbow trout (*Salmo gairdneri*). The virus and trout used in this study were obtained from a naturally occurring IPN epizootic in Colorado trout hatcheries and rearing units in 1968. IPN virus particles were found in large numbers in pancreatic acinar cells. Virus particles were associated most often with inclusion bodies composed of masses of virus particles and cell debris or large crystalline aggregations that were encircled by a single-layered membrane. Mature virus particles were found to average 57 nm in diameter and were hexagonal in profile.

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Cerini and Malsberger (1965) used electron microscopy to determine the size and morphology of infectious pancreatic necrosis (IPN) virus. The procedure leading to observation in the electron microscope involved isolation and concentration of the virus from rainbow trout gonad (RTG-2) tissue culture cells by ultracentrifugation, spraying of this concentrate on carbon and formvar-coated copper grids, and negative staining with phosphotungstic acid or shadowing with platinum–palladium wire. The platinum–palladium-shadowed particles were reported to average 29 nm (1 nm = 10^{-8} m); the negatively stained particles had an average size of 18.5 nm.

IPN virus has been reported by Wolf (1964) to pass a 50-nm filter readily, which is consistent with the size of 18.5 nm reported by Cerini and Malsberger (1965).

Moss and Gravell (1968, 1969) reported cell-associated IPN virus particles in RTG tissue culture cells to be 55 nm in diameter with a distinct hexagonal shape and symmetry, consistent with an icosahedron. Negatively stained cell-free virus particles were found to be 65 nm in diameter. Furthermore, Moss and Gravell (1969) showed that after density gradient ultracentrifugation, the 65-nm particles were at the same level in the centrifuge tube as was virus infectivity.

Observations of IPN virus in the tissues of infected trout are needed to clarify further the size and morphology of the virus. Such a study of IPN virus in rainbow trout (*Salmo gairdneri*) pancreatic cells is reported here.

Sampling and tissue preparation — The trout used in this study were obtained from a naturally occurring epizootic in several of Colorado's trout hatching and rearing units. The presence of IPN virus was determined in these fish by the serum neutralization technique, a

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standard method employed by the U.S. Bureau of Sport Fisheries and Wildlife in diagnosis of IPN disease.

The stomach and attached ceca were removed from fingerling rainbow trout dying of IPN disease, and subjected to primary fixation for 3 hr in 6% phosphate-buffered glutaraldehyde. Very small (ca. 1 mm^2) pieces of ceca and attached pancreatic tissue were then rinsed in *s*-collidine buffer and given secondary fixation in *s*-collidine buffered osmium tetroxide. Tissues were embedded in Dow Epoxy Resin using the procedure recommended by the manufacturer.

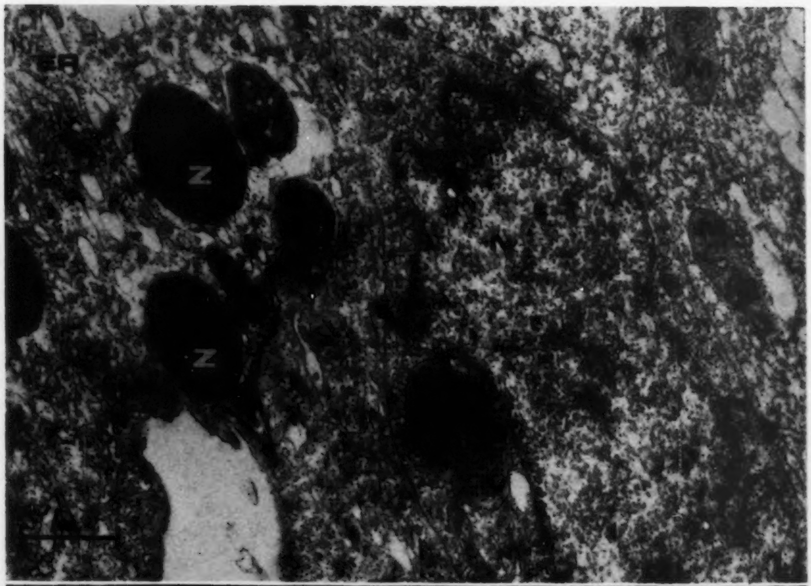
Electron microscopy — Thick and thin sections of pancreatic tissue were prepared using glass knives on a Sorvall MT-2 ultramicrotome. Sections 0.5–1 μ thick were picked up from the water-filled trough on the glass knife with a platinum wire loop, transferred to a glass slide, and fixed to the slide by heat. A saturated solution of orthotoluidine blue was used to stain the sections (Lynn, 1965). Stained sections were then examined by light microscopy for the presence of necrotic pancreatic tissue characterized by a general lack of distinctive tissue arrangement, by having inclusion bodies, and by a reduction in the number of cells containing zymogen granules. Thin sections were then made from those samples showing pancreatic necrosis. The thin sections were picked up on uncoated copper grids and stained with uranyl acetate and lead citrate. The grids were then carbon coated in a vacuum evaporator and viewed in a Zeiss E.M.9A electron microscope.

Results — IPN virus particles were found in large numbers in most of the pancreatic acinar cells that had some degree of pathological change. Virus particles were found most often in crystalline aggregations (Fig. 1–6). These aggregates were usually surrounded by a single membrane possibly derived from membranes of the Golgi complex or endoplasmic reticulum (Fig. 1–3). S. Sonstegard (personal communication) noted similar membrane-bound IPN virus aggregates in RTG cells.

The average diameter of 100 particles in thin sections of pancreatic acinar cells was 57 nm. The particles always appeared to be hexagonal in profile (Fig. 2, 3, 5). These results are consistent with those reported for IPN virus in RTG tissue culture cells by Moss and Gravell (1968, 1969).

IPN virus particles were found in the cytoplasm of acinar cells with only slight pathological changes such as swollen mitochondria and chromatin margination in the nuclei. Cells apparently were still functional at this stage, because the endoplasmic reticulum, Golgi apparatus, and zymogen granules appeared to remain intact (Fig. 1, 2).

Virus particles were found in greater abundance in pancreatic cells with more advanced pathological changes approaching necrosis. Inclusion bodies, resembling closely the myelin figures described by Swift and Hruban (1964), linked with at least some virus particles (Fig. 7) or inclusion bodies made up entirely of virus particles, were found regularly in pancreatic cells with advanced stages of degeneration. Inclusions consisting of large amounts of cellular debris and virus particles were seen regularly in pancreatic tissue (Fig. 4, 7, 8) and in the nearby fibroblasts (Fig. 6), which are abundant in the trout pancreas. Most of the inclusion bodies observed were from 1 to 6 μ in diameter. The acinar cells containing these inclusions tended to possess a reduced number of zymogen granules.



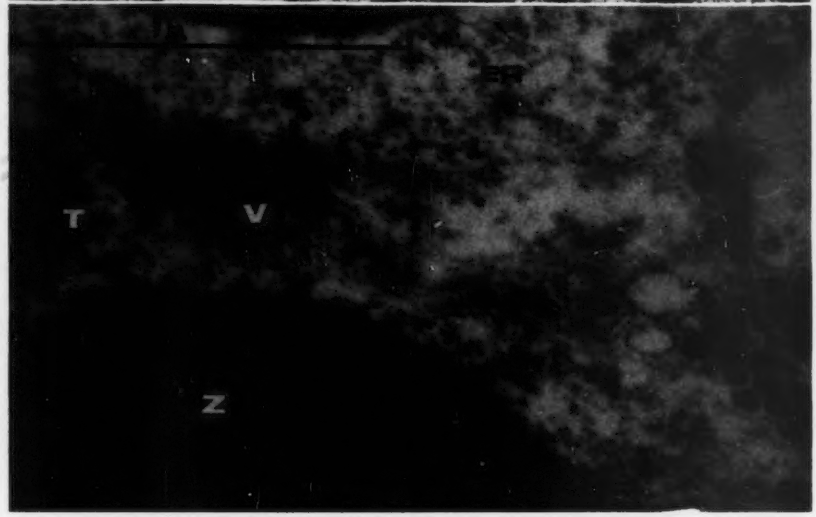


FIG. 1. IPN-infected rainbow trout (*Salmo gairdneri*) pancreatic acinar cells. Except for the presence of IPN virus (V) and some swelling of the endoplasmic reticulum the cells are essentially normal. N = nucleus; M = mitochondria; Z = zymogen granules; ER = endoplasmic reticulum; V = virus. ×15,000.

FIG. 2. A higher magnification of an IPN virus lattice structure (V) near a zymogen granule (Z) in a pancreatic acinar cell of a rainbow trout. A microtubule (T) is present. ×61,700.

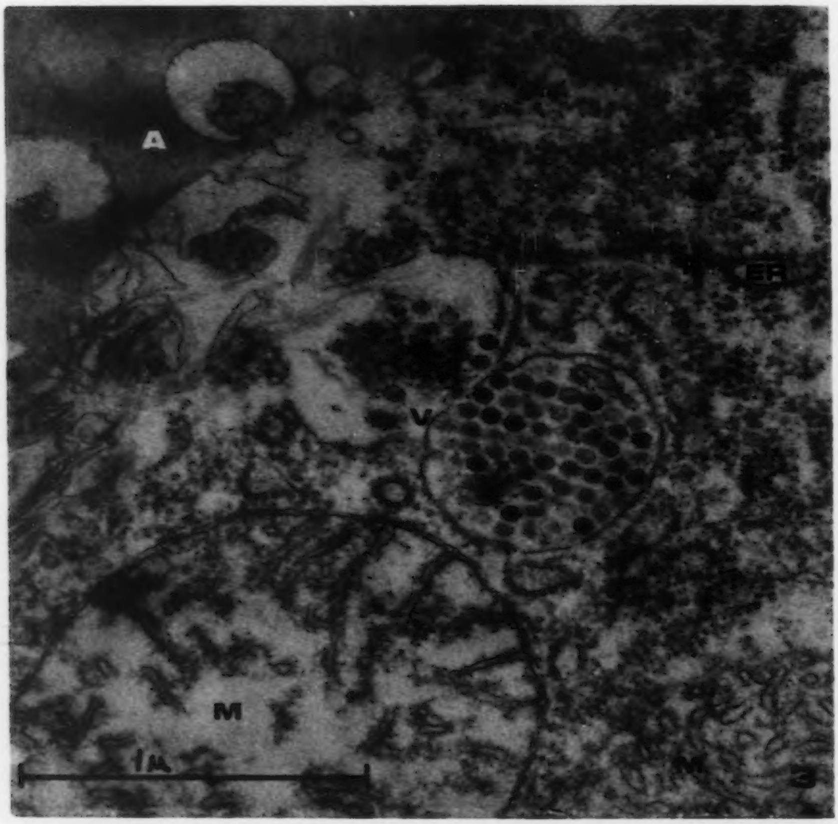
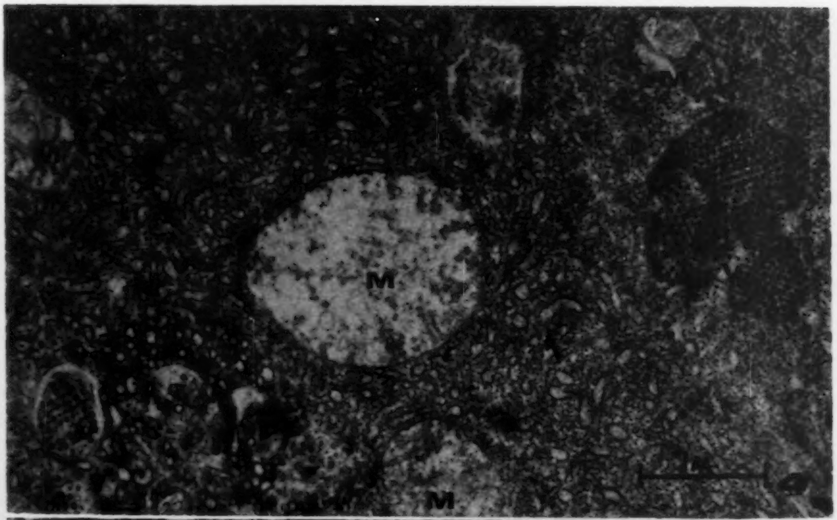


FIG. 3. Rainbow trout pancreatic acinar cell showing swollen and degenerate mitochondria (M), an amorphous glassy material (A), and two membrane-bounded structures containing virus particles (V). Swollen endoplasmic reticulum (ER) and associated ribosomes are abundant. A cytoplasmic microtubule (T) that is approximately 25 nm is present near the virus structures. ×53,400.



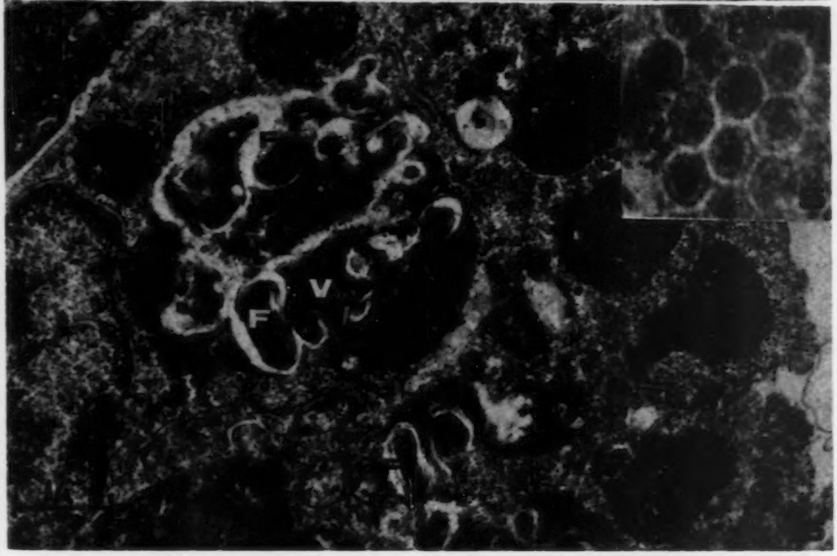


FIG. 4. Rainbow trout pancreatic acinar cell. Two IPN virus lattice structures (V) and an inclusion body (I) composed of randomly arranged virus particles and degenerating cytoplasm are present. Normal and swollen mitochondria are also present. ×19,500.

FIG. 5. A higher magnification of the enclosed area of Fig. 4. ×128,400.

FIG. 6. Rainbow trout fibroblast that was adjacent to several acinar cells. Several large inclusion bodies composed of myelin figures (F) and masses of IPN virus (V) are shown. ×15,400.

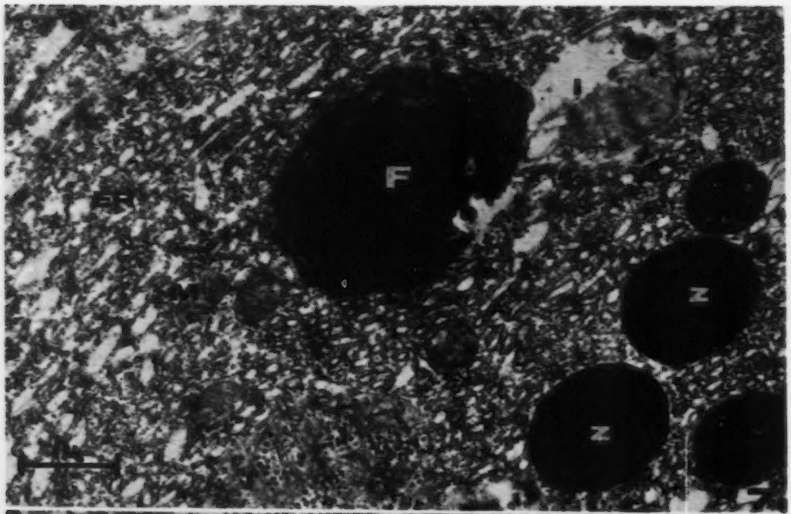


FIG. 7. Rainbow trout pancreatic acinar cell containing a large myelin figure (F) that appears to have been derived from granular ER. Inclusion bodies (I) composed of masses of IPN virus particles and degenerating cytoplasm are present. Other structures shown are zymogen granules (Z), normal mitochondria (M), and granular endoplasmic reticulum (ER). ×15,000.

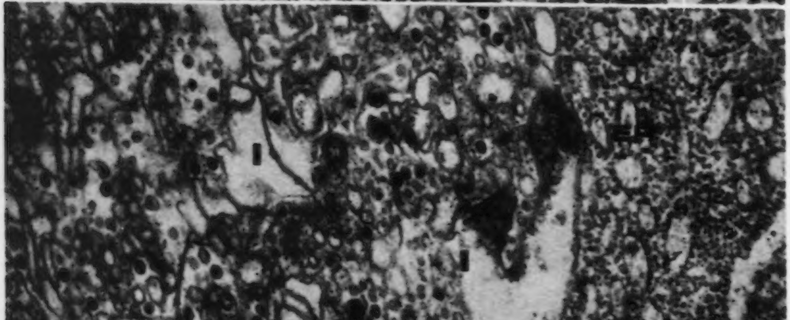


FIG. 8. A larg epancreatic inclusion (I) composed of degenerating cytoplasm and irregularily arranged IPN virus particles. Essentially normal cytoplasm containing a mitochondrion (M) and granular ER is separated from the inclusion by a single-layered membrane (arrow). ×43,000.

Discussion — The reasons for differences in size reported for IPN virus are not known. The theory that different strains of IPN virus may be different in size is a possible, but doubtful, answer. The most probable answer is that Cerini and Malsberger (1965) did not observe IPN virus particles. Moss and Gravell (1969) gave excellent evidence that the particles reported in their study were indeed IPN by showing IPN particles to be at the same level as infectivity after density-gradient ultracentrifugation.

The inclusion bodies of IPN vary in composition and size, and tend to range among three basic types. The first type consists mostly of virus particles in a crystalline arrangement (Fig. 1–6) whereas the second type is composed of randomly arranged virus particles intermixed with masses of degenerating cytoplasm (Fig. 4, 8); a third type is made up of myeloid membranes (Fig. 6, 7). Swift and Hruban (1964) demonstrated similar myelinlike inclusion bodies in rat pancreas cells after exposure to toxic substances. The suggestion was made that the myelin figures represented the response of a living cell to a toxic stimulus. Cell death or necrosis would result only secondarily, if the process of inclusion formation and digestion could not ultimately cope with the toxic stimulus. Wood et al. (1955), using light microscopy, described pancreatic inclusion bodies of IPN as small, blue, usually oval bodies surrounded by a clear halo. Malsberger and Wolf (1966) and Wolf (1966) gave similar descriptions of pancreatic inclusion bodies. Many of the inclusions observed in this study were composed of large amounts of virus and could be expected to stain blue with hematoxylin. However, inclusions composed of virus crystals were generally much smaller than the other types of inclusions, being less than 2 μ in diameter. Myelin figures and inclusions consisting of cell debris and virus may represent the inclusions normally seen in paraffin preparations of IPN-infected pancreatic tissue. They are generally larger, ranging from 1 to 6 μ in diameter. The degree of basophilia of the inclusions could be expected to vary with the relative amount of viral RNA or ribsomal RNA present. Some inclusions, though not all, were surrounded by a clear halo as described by Wood et al. (1955).

Moss and Gravell (1969) reported elongated tubules that were doughnut-shaped in cross section. These tubules were smaller in diameter (45 nm) than mature virus particles (55 nm). The tubules were found in RTG, fathead minnow (FHM), and bluegill (BGL) tissue culture cells 5–7 hr after infection. They were occasionally seen in association with mature virus particles. No such tubules were seen in the study reported here. An occasional cytoplasmic microtubule approximately 25 nm in diameter was found in association with virus structures (Fig. 2, 3). Moss and Gravell (1969) suggested that the 45-nm tubules may be spindle fibers coated with viral antigen or that they may be aberrant virus forms. The latter suggestion may be the case as tissue culture systems may not be entirely representative of the occurrence in intact fish. The possibility also exists that the tubules are present early in the infected cells of the host fish and later disappear or are changed. Infected trout fry for this study were taken late in the infection as the classical signs of IPN disease were apparent. Infected cells may not have contained tubules for that reason.

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Maintenance of the Life Cycle of *Ligula intestinalis* in the Laboratory

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Techniques for the maintenance of the life cycle of *Ligula intestinalis* in the laboratory are described. Fertile eggs were produced by adult cestodes 72 hr after ducklings were fed with infective plerocercoids. Eggs were extracted by sieving and washed by decanting prior to incubation in water for 10 days at 25 C. A bright light stimulated developed eggs to hatch, and the emergent coracidia were fed to the copepods *Diaptomus fragilis* and *Mesocyclops leuckarti*. Infective procercoids were present in the haemocoel of copepods after 10 days at 25 C. Plerocercoids developed in tropical cyprinid fish, e.g., *Danio malabaricus*, which were infected by exposure to infective copepods.

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The life cycle of *Ligula intestinalis* with crustacean, fish, and bird hosts enables host–parasite relationships to be studied in three different types of animal. Of particular interest is the comparison that can be made between the plerocercoid of *L. intestinalis*, which has been reported from several species of cyprinid fish, and the plerocercoid of another common pseudophyllidean tapeworm, *Schistocephalus solidus*, which is host specific to one species, the threespine stickleback *Gasterosteus aculeatus* (Bråten, 1966). To carry out such studies it is essential to be able to maintain these worms throughout their life cycles in the laboratory. The maintenance of *S. solidus* in the laboratory has already been described (Orr and Hopkins, 1969). This note details the host and procedural differences necessary to maintain the life cycle of *L. intestinalis*.

Infective plerocercoids of over 500-mg fresh weight, obtained from the body cavity of fish (roach: *Rutilus rutilus*), were force fed to day-old ducklings. Faeces were collected from 72 to 168 hr after infection. The eggs were extracted by sieving, washed, and placed in water in crystallizing dishes. After 24 hr in an incubator at 25 C the eggs were washed and replaced in the incubator in darkness for a further 9 days. After 10 days at 25 C a bright light stimulated the eggs to hatch.

Two species of copepod, *Diaptomus fragilis* and *Mesocyclops leuckarti*, serve as copepod hosts for the procercoid stage, which is infective 10 days after entry into the haemocoel at 25 C. *Cyclops agilis*, a good host for *S. solidus*, is not a suitable host for *Ligula*, as the oncosphere degenerates shortly after entry into the haemocoel.

Several species of cyprinid fish may be infected by exposure to infected copepods. However, the establishment and growth of plerocercoids in tropical cyprinids, which are easily maintained, make these fish suitable laboratory hosts for long- or short-term work. The following tropical fish have been infected in the laboratory: Schuberti barbs (*Barbus sachsi*), Giant Danios (*Danio malabaricus*), and White Cloud Mountain minnows (*Tanichthys albonubes*). The Giant Danios, which reach a size of 10–12 cm, are ideal as laboratory hosts. Plerocercoids from multiple infections in these fish possess genital primordia after 60 days at 25 C.

Work previously limited to field observations on the growth rate and castration of infected fish, the growth of the plerocercoid, and immunological reactions of the host, can be complemented with defined conditions in the laboratory.

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Age and Growth of Broad Whitefish, *Coregonus nasus*, in the Mackenzie and Coppermine Rivers, N.W.T.

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Muth, K. M. 1969. Age and growth of broad whitefish, *Coregonus nasus*, in the Mackenzie and Coppermine rivers, N.W.T. J. Fish. Res. Bd. Canada 26: 2252–2256.

Broad whitefish, *Coregonus nasus*, from the Coppermine River differ in both rate of growth and longevity from those in the Mackenzie River. Whitefish from the Mackenzie River are faster growing during the first 6 years of life but have a shorter life span than whitefish from the Coppermine River. Mackenzie River data correspond favorably with age and growth of broad whitefish reported from several rivers in the USSR. Differences in environmental factors such as water temperature, ice conditions, and food species availability are suggested as causes for fish growth differences in the two rivers.

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Arctic fishery surveys during the summers of 1956, 1957, and 1961 provided age and growth information on broad whitefish, *Coregonus nasus*, from the Mackenzie and Coppermine rivers. Previous information concerning this species in Canada is sparse and largely concerned with taxonomy or distribution.

In Canada, the broad whitefish is confined to fresh or brackish waters near coastal areas in the arctic west of Bathurst Inlet (67°N; 109°W) and it is not commercially exploited or a contributor to the sport fishery. However, the species is commercially fished in Russian waters and more detailed information on growth is available from this fishery. Meaningful data comparisons between Russian and Canadian broad whitefish are possible because no subspecific distinction exists (Walters, 1955).

Materials and methods — Samples for this study consisted of 564 fish from the Mackenzie River and 263 fish from the Coppermine River. They were obtained by gillnets with stretched mesh sizes of 1½, 2½, 3½, 4, 4½, and 5½ inches, each net being 50 ft long and 6 ft deep. Six nets, one of each mesh size, were tied into a gang and normally set at a right angle to the shore line on the Mackenzie River. Coppermine River current and width prevented the use of net gangs and only individual nets set at a right angle to the shore line were used. Net sets in both rivers were usually not deeper than 8 m.

Specimen weights were converted to metric equivalents from pounds and fractions of pounds recorded in the field. Fork lengths were recorded to the nearest millimeter. Most fish were dissected and sexes tabulated but sexual maturity was not recorded because the fish were captured in summer and would not ripen until fall.

Scale samples were collected from an area below the anterior edge of the dorsal fin and were used for age analysis and back calculations of annual length increments. Scales were relatively easy to read and the same ages were recorded by several investigators in over 75% of the readings. Scale readings that were not in complete agreement for all investigators usually did not differ by more than ±1 annulus. These were assigned the ages that the majority of investigators agreed upon.

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Length–weight relationships were calculated for fish from each river by the formula:

$$\log W = \log c + n \log L$$

where W is weight in grams, L is length in centimeters, and c and n are computed constants (Rounsefell and Everhart, 1953).

Few fish under 4 years of age were taken by the gillnets but growth was estimated for the ages not represented. Scales were projected at a magnification of 20× and measurements from the foci to the anterior sides of the scales were tabulated. Regression equations for the relationship between average fork length and average scale measurements were computed for fish from both rivers (Fig. 1). Annual length increments were then calculated by tabulating scale measurements from the foci to given annuli and substituting these values in the previously determined equations.

Growth — The main age-classes of broad whitefish taken in the Coppermine and Mackenzie rivers were age V through age XIV (Table 1). No fish

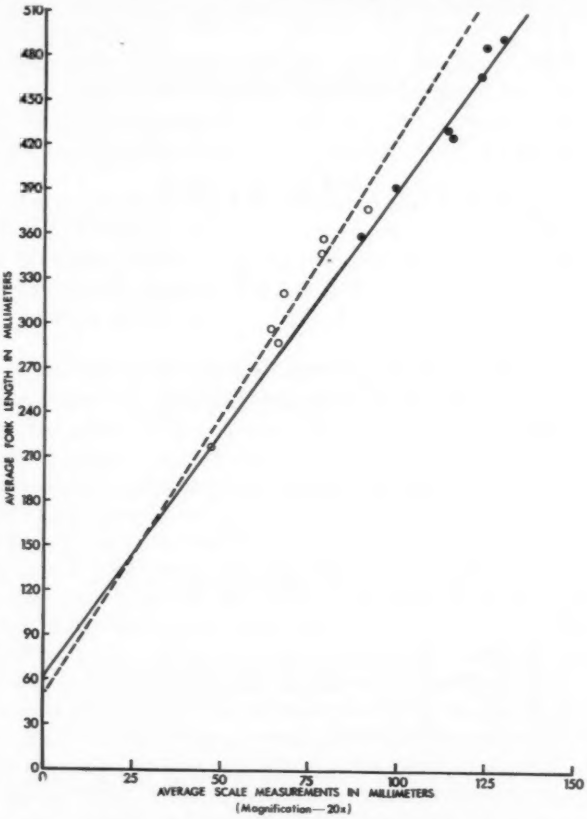

FIG. 1. Relationships between fork lengths and projected scale measurements of broad whitefish from Coppermine and Mackenzie rivers. ●—● MacKenzie fish, $Y = 62.04 + 3.29X$; ○- - -○ Coppermine fish, $Y = 52.14 + 3.75X$. Regression equations are computed from 70 Coppermine and 161 Mackenzie specimens between 5 and 11 years of age.

TABLE 1. Sizes at capture of broad whitefish from the Coppermine and Mackenzie rivers, and back-calculated lengths at the end of each year of life. Back-calculations were based on annuli measurements. Also shown are weights of 269 and 104 broad whitefish from the Kara and Kolyma rivers in the USSR.

	Coppermine River					Mackenzie River					Kara River	Kolyma River
	Avg size at capture			Calculated size at end of year of life		Avg size at capture			Calculated size at end of year of life			
Age	No. fish	Wt (g)	F.L.[a] (mm)	No. fish	F.L. (mm)	No. fish	Wt (g)	F.L. (mm)	No. fish	F.L. (mm)	Avg wt (g)	Avg wt (g)
1	–	–	–	72	94.18	–	–	–	113	126.26	40	–
2	–	–	–	72	128.64	4	85	184	113	189.95	127	–
3	–	–	–	72	167.19	6	185	244	113	255.16	265	–
4	1	113	208	72	202.63	12	444	317	113	315.83	347	376
5	12	279	290	72	232.40	39	640	355	113	362.91	639	541
6	19	297	285	64	267.77	64	783	374	93	397.88	813	964
7	29	338	308	59	293.42	92	1060	406	63	427.69	1063	1226
8	40	408	328	50	320.49	69	1279	432	44	442.40	1893	1809
9	43	501	352	38	347.60	66	1656	464	20	468.85		2560
10	30	691	377	26	366.13	67	1595	462	12	487.27		
11	21	672	376	11	400.22	65	1869	485	7	497.27		
12	12					49						
13	10					20						
14	19					10						
15	11					1						
16	9											
17	4											
18	3											
Total	263					564						

[a] F.L. = fork length.

older than age XV were taken in the Mackenzie River, whereas several fish in age classes XVI–XVIII were taken in the Coppermine River. Calculated annual increments in length for the first 6 years of life were notably lower for the Coppermine fish than for fish from the Mackenzie River.

Length–weight relationships calculated for fish between 160 and 600 mm in length from the Coppermine and Mackenzie rivers are

$$W = .004\ L^{3.28} \text{ and } W = .003L^{3.41}$$

respectively. These equations were derived from combined male and female data after initial testing indicated no significant sexual difference ($P < 0.05$) in length–weight relationships for fish within a given drainage.

A comparison of ages for similar length fish from the two rivers indicates that Coppermine fish may be as much as 4 years older than Mackenzie fish for a given length-class.

Growth in weight of broad whitefish in the Mackenzie River compares favorably with growth to age VII in two Russian rivers whereas growth of the Coppermine fish compares poorly for all ages. Length comparisons are not possible because Russian data are in terms of total length instead of fork length.

The usual sizes of broad whitefish taken in the Russian fishery are 50–60 cm in length and 2–4 kg in weight, but some as large as 16 kg are reported (Berg, 1948; Nikolsky, 1954). Unconfirmed reports indicate Canadian broad whitefish may reach 10 kg in weight but 5.5 kg (J. G. Hunter, unpublished data) is the greatest documented weight known to this author.

Discussion — One cause of the different growth rates for broad whitefish in the Mackenzie and Coppermine rivers might be the existence of a slow- and a fast-growing form of the species. Dryagin, Averintsev, and Menshikov have reported slow-growing and fast-growing forms in the Kolyma, Lena, and Ob rivers respectively but the reasons for this within-drainage growth difference (be they genetic or environmental) are not stated (Berg, 1948). Other Russian writers have concluded that, at least in some rivers, distinct forms do not exist. Mackenzie and Coppermine river data are not extensive enough to confirm or deny the existence of different forms and other reasons for the growth differences seem more logical.

The environmental factors water temperature and food availability are suggested as the primary causes of growth differences between the Coppermine and Mackenzie fish. The Mackenzie River begins to freeze in mid-October and usually remains ice-covered until the last week of May (Cooper, 1967). Data provided by the Canada Department of Energy, Mines and Resources, Inland Waters Branch, indicate water temperatures are slightly above 0 C under the ice and increase after break-up to approximately 15.5 C in July and August, followed by a rapid decline in September. The Coppermine River water temperature patterns are similar to the Mackenzie temperatures for the fall, winter, and spring but seldom exceed 10.0 C in the summer. Freeze-up begins about the 1st or 2nd week of October and break-up does not occur until the middle of June (Usher, 1965). The net result is an open-water growing season approximately 1 month shorter in the Coppermine River, and a maximum summer water temperature of nearly 5.5 C less.

The availability of whitefish food, such as molluscs, insects, or crustaceans, in the two rivers was not determined but insects seemed to be abundant in both. The number of species of molluscs is nearly twice as great in the Mackenzie River as in the Coppermine (A. H. Clarke, personal communication) and may result in a greater variety of food sources at certain times of the year. This could be another factor contributing to growth differences.

Acknowledgments — The author wishes to express his gratitude to Dr J. G. Hunter for making the data available from arctic fishery surveys that he conducted and for critically reviewing the manuscript. Dr C. J. Kerswill and Dr J. W. Wacasey also read the manuscript and offered helpful suggestions. Miss B. A. von Nide and Miss S. T. Leach assisted with aging and back-calculations.

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Heading and Gutting of Capelin With Special Hand Shears

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Sepic, K., and W. A. MacCallum. 1969. Heading and gutting of capelin with special hand shears. J. Fish. Res. Bd. Canada 26: 2257–2259.

The hand shears traditionally used for heading and gutting sardines in Mediterranean canneries were shown to increase the heading rate for capelin to about four times that prevailing in Newfoundland plants, where simple knives are used. The shears could greatly increase production efficiency in canning capelin, small herring, smelt, and other inland species at small Canadian plants and could be useful in experimental laboratories and households, especially those of sports fishermen and fish food enthusiasts.

Received February 5, 1969

This note discusses the possible application in Newfoundland and other Canadian provinces of special hand shears (Fig. 1) used successfully in Mediterranean countries[2] for heading and gutting small fish prior to canning. For example, use of the shears instead of a knife or scissors would greatly increase productivity in canning beach-spawning capelin (*Mallotus villosus*) at small plants. An increase in productivity in this operation is urgently needed. Although headed, gutted capelin may be dried and lightly smoked to produce a first-class canned product (Horne et al., unpublished data; Lantz, 1966), this item is not yet widely known. Any efforts expended to establish a general market will be met by strong competition. Low cost of production would help to make the canned capelin competitive and use of the Mediterranean-style shears would make a substantial contribution.

The uncertainty of the length of the period required to develop a satisfactory market for a good product provides excellent reason for keeping capital costs for a capelin canning operation low. At this stage of development, however, good use cannot be made of automatic heading and gutting machinery and

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[2] Shears, believed to be constructed on the same principle, are reported to have been in family use on the "South Shore" of Nova Scotia for many years (D. A. Eisenhauer, personal communication), presumably for preparing herring for pickling.

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heading with a knife or conventional scissors, along with gutting as an additional step to ensure removal of sand from the gut,[3] is too expensive.

In several Newfoundland plants, heading is done presently with simple knives and one person heads and guts an average of only 30 lb of fish per hour. In contrast, in the sardine (*Clupea pilchardus*) industry in many Mediterranean countries in which the special shears (Fig. 1) are used, the average production per worker per hour is 45–60 kg (100–132 lb) of fish (32 per kg, 14.5 per lb), and many women exceed this rate by 50%. These greatly improved rates suggested a need for a trial of the shears on capelin; consequently samples of the shears were obtained for testing in Newfoundland.

FIG. 1. Hand shears shown in open position.

Two experiments were performed in St. John's, one during June 1967, at the College of Fisheries, and the other during June 1968, at the Technological Unit of the Fisheries Research Board of Canada. The capelin numbered 12–14 per pound. In both experiments the persons participating had no previous experience in heading and gutting capelin with these shears. The two experiments showed that the shears with shape adapted to the anatomy of sardines can be used for capelin without any change in design. With the shears properly placed, one cutting stroke (Fig. 2) coupled with a single move of the closed cutting edges in the direction of the head of the fish removed the head and almost all the guts from the fish; the advantage of the shears over the knife or simple scissors was shown to be in the gutting step. Untrained persons using the special shears headed and gutted 120–130 lb of capelin per hour on the average, or about four times as many as workers in Newfoundland plants using knives. The appearance of the cut was very satisfactory for both male and female capelin.

There are some anatomical differences between the sardine and the capelin that should be noted by the prospective user of these shears. The sardine has

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[3] Capelin caught during their beach-spawning period frequently have sand in their stomachs making gutting necessary, whereas in the New Brunswick and Maine sardine canning industry, the small herring need to be headed only, for which purpose simple scissors are used with great skill and efficiency.

FIG. 2. Heading and gutting capelin in a single operation using the hand shears illustrated in Fig. 1. The cutting edges of the shears rest on the bony part of the pectoral fins. A single cutting stroke coupled with movement of the closed cutting edges in the direction of the head removes the latter and almost all the guts from the fish.

small pectoral fins behind the gill covers, and the capelin much larger ones. In capelin the point of attachment of these fins at the pectoral girdle is as far forward as the posterior tip of the gill covers. With the sardine, it is possible to make a clean dorsal–ventral cut with the shears and leave the pectoral fins on the body, and the gill covers with the severed head. However, if the shears are held the same way behind the gill covers of the capelin the cutting edges cross the anterior parts of the pectoral fins and these are difficult to sever. With these fish, placing the cutting edges of the shears under or even over the less bony part of the pectoral fins permits the head, with attached pectoral girdle and fins, to be severed easily. This cut can be made dorsally–ventrally as with the sardine, or obliquely from the belly to the back if maximum yield is desired. With up to three fish in one hand, a worker can move the shears, held in the other hand, into position under or over the pectoral fins for a quick, efficient operation.

Besides capelin, small herring (also available off Canada's Atlantic Provinces) and smelt from the Great Lakes region are of similar size to sardines and, along with small mackerel, possibly could be processed with the same shears. Their simple construction and easy use could result in their becoming a normal tool in processing plants, experimental laboratories, and many households, particularly those in which sports or other whole fish are enjoyed.

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Triaenophorus crassus in Arctic Lampreys of the Northwest Territories, Canada

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Buchwald, D. G., and J. R. Nursall. 1969. *Triaenophorus crassus* in arctic lampreys of the Northwest Territories, Canada. J. Fish. Res. Bd. Canada 26: 2260–2261.

Unencysted plerocercoids of *Triaenophorus crassus* Forel are recorded from the coelom of arctic lampreys (*Lampetra japonica* Martens) from Great Slave Lake, N.W.T. This is a new host record.

Received March 13, 1969

During the course of an investigation on the arctic lamprey (*Lampetra japonica* Martens) in Great Slave Lake, Canada, plerocercoids of *Triaenophorus crassus* Forel, 1868, were found. They were present in 12.5% of the 112 adult lampreys examined.

Lampetra japonica is a new host record, although *T. crassus* has been recorded from the sea lamprey, *Petromyzon marinus* (Wilson and Ronald, 1967). Earlier records are summarized in Lawler and Scott (1954).

The plerocercoids were found unencysted in the coelom, frequently attached to the liver and occasionally to the gonads. Unencysted plerocercoids are commonly found in the muscles of European fishes and have been recorded from the muscles of yearling coregonines in North America (Miller, 1952).

The plerocercoids were probably acquired through ingestion of procercoids in infected *Cyclops* by the ammocoetes. Plerocercoids of some pseudophyllids (e.g., diphyllobothrids — Vik, 1964) can reenter the musculature when ingested by an animal other than a suitable definitive host. The observations of scolices, or partially disintegrating "adults," in the intestines of sea lampreys (Wilson and Ronald, 1967) suggest that *T. crassus* cannot.

The identification was made by Dr J. C. Holmes, Department of Zoology, University of Alberta, Edmonton, Alta.

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CORRECTIONS TO RECENT PUBLICATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA

Journal of the Fisheries Research Board of Canada

VOLUME 23, NO. 2 — paper by K. Radway Allen: *A method of fitting growth curves of the von Bertalanffy type to observed data.*

Page 172: line 21, the equation should read

$$``a_3 = L_\infty e^{kt_0}[t_0 e^{kt_0}\Sigma e^{-2kt} - e^{kt_0}\Sigma t e^{-2kt} - t_0 \Sigma e^{-kt} + \Sigma t e^{-kt}]"$$

Page 173: line 5, the equation should read

$$``a_3 = L_\infty Z^2(t_0 E - H) + L_\infty Z(G - t_0 C) = c_1 = r"$$

VOLUME 24, NO. 11 — paper by Ian E. Efford: *Temporal and spatial differences in phytoplankton productivity in Marion Lake, British Columbia.*

Page 2283: Abstract, line 12, "0.0009%" should read "0.09%."

Page 2283: Introduction, line 6, "energy" should read "carbon."

Page 2290: Fig. 4, abscissa, "light (g cal/sec)" should read "light (g cal/cm^2 per sec)."

Page 2292: line 12, "photosynthesis" should read "photosynthetic contribution."

Page 2293: Fig. 5, ordinate should read "mg C/m^3 per day."

Page 2305: line 28, "0.0009%" should read "0.09%".

VOLUME 25, NO. 4 — paper by Isobel Mackay and G. Power: *Age and growth of round whitefish (Prosopium cylindraceum) from Ungava.*

Page 659: Para. 3, line 2, should read "$w = cn^{L}$."

Page 662: Table III, title should read "Calculated fork length . . ."

Page 662: text table, number of specimens under 12+ should read "5."

VOLUME 25, NO. 9 — paper by R. R. Parker, Z. Kabata, L. Margolis, and M. D. Dean: *A review and description of Caligus curtus Müller, 1785 (Caligidae: Copepoda), type species of its genus.*

Page 1958: Fig. 35, caption should read "Male 2nd antenna, ventral."

VOLUME 25, NO. 10 — paper by John G. Stockner: *Algal growth and primary productivity in a thermal stream.*

Page 2045: Para. 4, line 9, should read ". . . photosynthetic quotient (P.Q.) were 1.0, ($\frac{+\Delta O_2}{-\Delta CO_2}$ = . . ."

Printed in Canada

VOLUME 26, No. 2 — paper by C. D. McAllister: *Aspects of estimating zooplankton production from phytoplankton production.*

Page 203: line 4, should read "... (unpublished) obtained data..."

Page 204: Para. 4, line 5, should read "... mean production, $\bar{p}$."

Page 207: Fig. 4, caption, line 10, should read "3, 2, 1, respectively..."

Page 217: Add to References:

TABATA, S. 1960. Characteristics of water and variations of salinity, temperature, and dissolved oxygen content of the water at Ocean Station "P" in the Northeast Pacific Ocean. J. Fish. Res. Bd. Canada 17: 353–370.

TABATA, S., AND L. F. GIOVANDO. MS, 1963. The seasonal thermocline at Ocean Weather Station "P" during 1956 and 1959. Fish. Res. Bd. Canada MS Rept. (Oceanog. Limnol.) 157. 7 p.

Page 218: Equation 1 should read

$$\text{“} \int_{t=0}^{t=t_N} (I')dt = \bar{g}\, P_S \, \frac{(1-e^{-(\bar{g}+r)t_N})\text{”}}{(\bar{g}+r)}$$

Page 219: Para. 1, lines 8 and 9 should read

$$\text{“}I_t = g_t P_t = g_S\,(1-at)P_t$$

$$= g_S P_S\,(1-at)\,e^{-(\bar{g}+r)t}\text{”}$$

Page 219: Equation 4, second line should read

$$\text{“} = g_S P_S\,(1 + (1-at)_N\, e^{-(\bar{g}+r)t_N})\tfrac{1}{2}\text{”}$$

Page 220: Line 1 of the expression giving "M–1" the numerator of the first term on the right-hand side of the equation should read

$$\text{“}1 + (1-at_N)\, e^{-(\bar{g}+r)t_N}\text{”}$$

Page 220: Para. 2, line 7, should read "... $\bar{g} = 0.02$; ..."

VOLUME 26, No. 6 — paper by D. V. Subba Rao and T. Platt: *Optimal extraction conditions of chlorophylls from cultures of five species of marine phytoplankton.*

Page 1630: Table 3, last line, should read "*Phaeodactylum*..."

Recent Articles on FRB Investigations Published in Other Periodicals

Studies No.

1323. Ackman, R. G., M. Kates, and R. P. Hansen. 1969. Diastereoisomeric composition of pristanic acids of marine and terrestrial origin. Biochim. Biophys. Acta 176: 673–675.

1324. Anderson, J. M., and M. R. Peterson. 1969. DDT: sublethal effects on brook trout nervous system. Science 164: 440–441.

1325. Idler, D. R., and B. Truscott. 1969. Production of 1α-hydroxycorticosterone *in vivo* and *in vitro* by elasmobranchs. Gen. Comp. Endocrinol. Suppl. 2: 325–330.

1326. Seki, H., K. V. Stephens, and T. R. Parsons. 1969. The contribution of allochthonous bacteria and organic materials from a small river into a semi-enclosed sea. Arch. Hydrobiol. 66: 37–47.

Copies of individual reprints, if available, may be obtained from the authors or Board establishments through which the investigations were conducted.

Reprints of these articles are included in an annual volume of "Studies," composed of two parts. These are distributed to a selected mailing list. (Articles numbered 1235 to 1262 appeared in the Studies for 1968, Part 2.)

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